

***This presentation should have included all images analyzed in the paper plus some images (from CG/Chat-Cre-ZsGreen) mice for another project  
Due to size limit, it is printed to pdf.***

Rationale:

Previous report in cancer research indicated that VEGFR1 activation led to increased thermal and mechanical sensitivity via TRPV-1 and TRPA-1 mediated pathways, respectively.

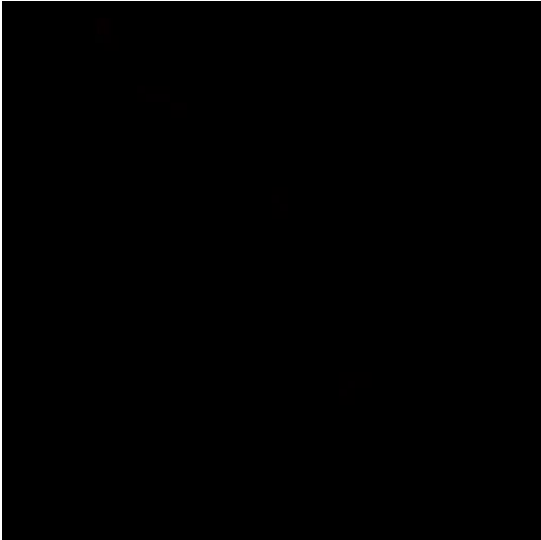
Western blot analysis showed that VEGFR1, but not VEGFR2, was significantly upregulated in the lysates of ipsilateral L3–L4 lumbar DRGs isolated from tumor-bearing mice as compared with sham-treated animals.

**Question: Does the expression level of VEGFRs, TRPV1 and TRPA1 change in DRG sensory neuronal somata in response to bladder VEGF<sub>A</sub> instillation?**

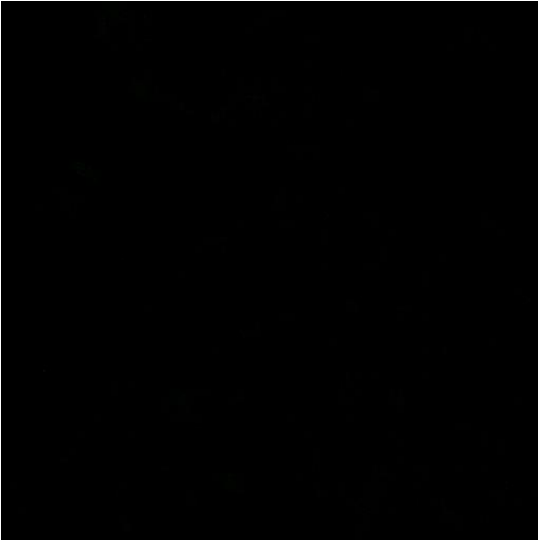
Experimental design: Littermates receive bladder instillation of either VEGF<sub>A</sub> or saline. Immunostaining for VEGFR1, R2, TRPV-1, and TRPA1 in L5/L6-S2 DRG.

The 488 channel was reserved for ZsGreen signal, 594 channel was used for anti-VEGFR1, anti-VEGFR2, or anti-TRPV-1. DAPI was used to reveal the cell nuclei.

Rosa-ZsGreen +/+ mice  
(no naïve ZsGreen)

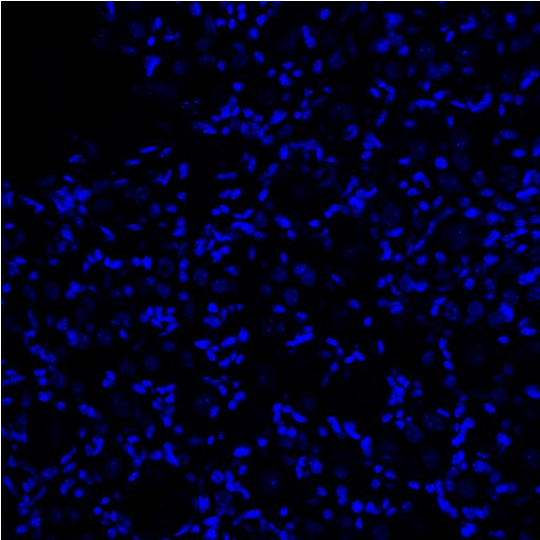


Non-primary

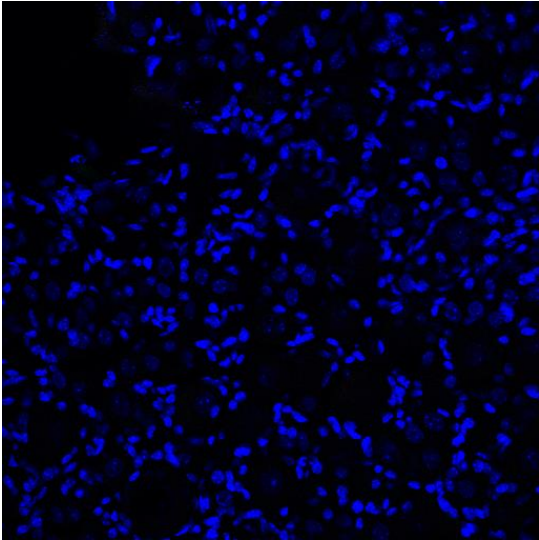


Little autofluorescence, used the same setting to document antibody staining

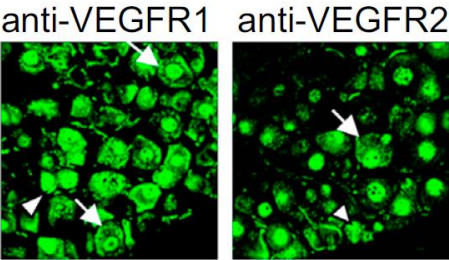
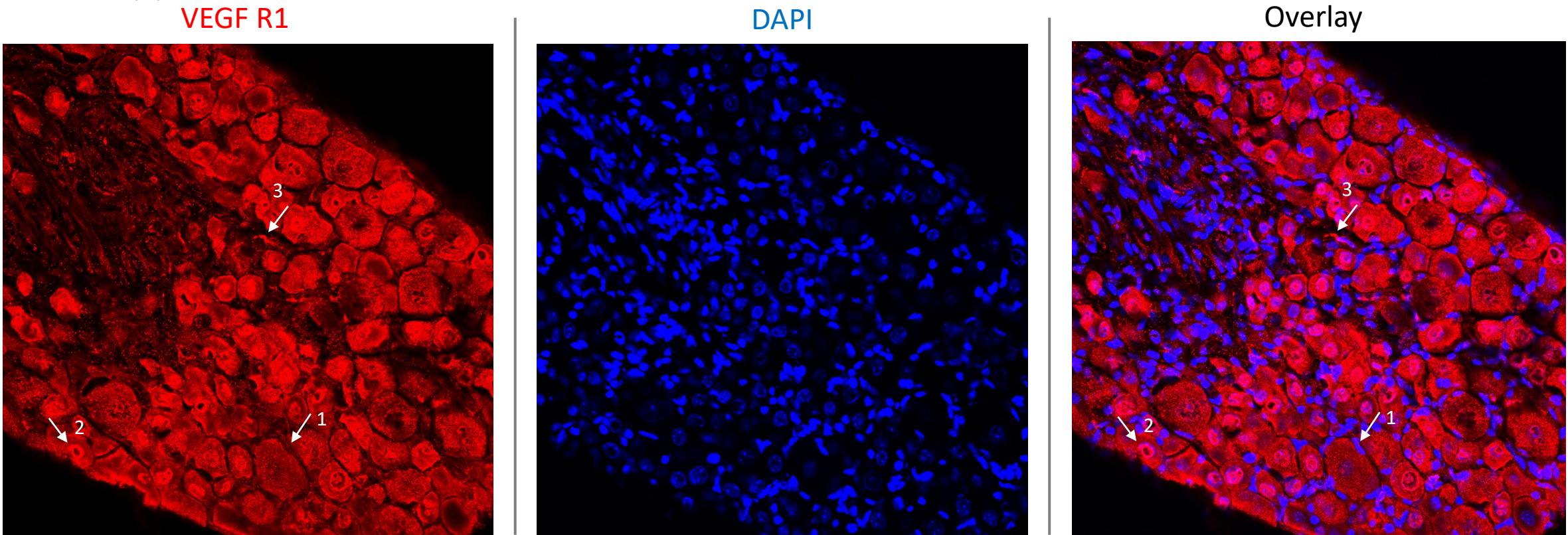
DAPI



Overlay



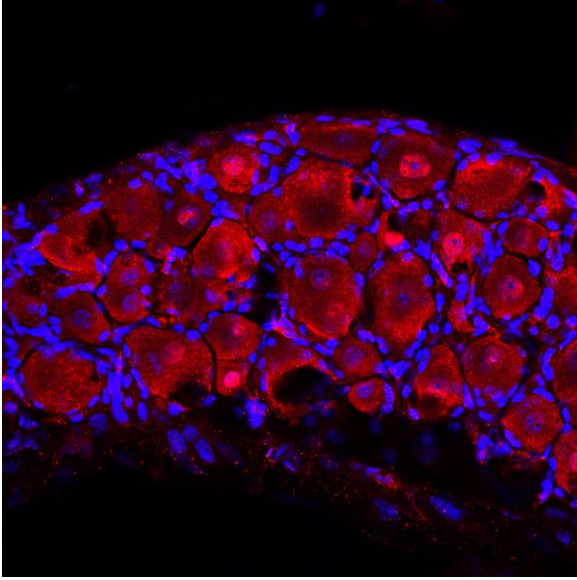
Antibody against VEGFR1 exhibits broad staining in DRG neurons and potentially satellite glia cells (1), with strong perinuclear staining (2). The VEGFR1 signal is also detected in the cytosol of small diameter neurons (3).



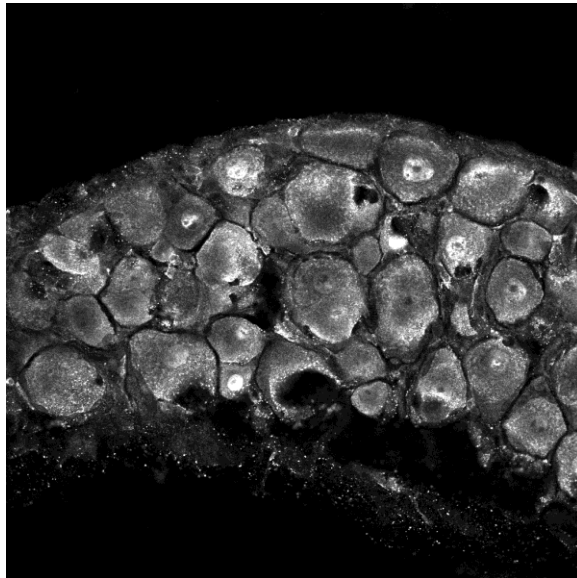
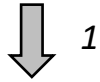
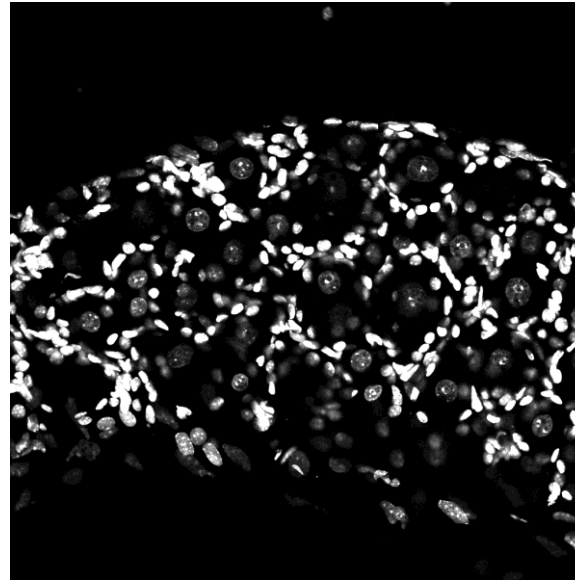
20190328 RGs/PBSVR1  
RG female\_R0165\_40x\_1  
Saline Instillation



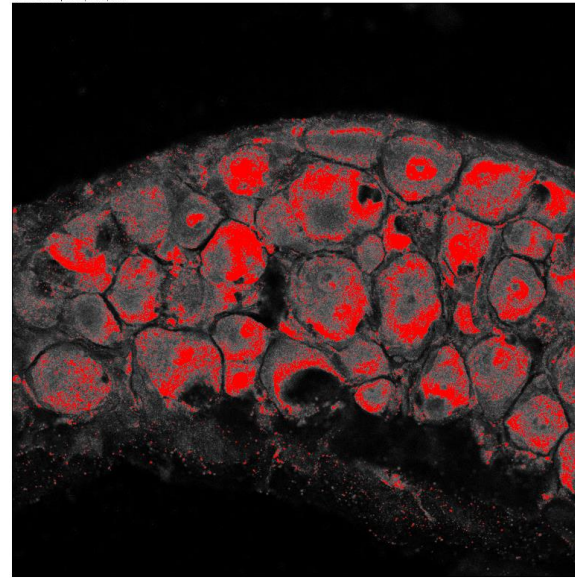
Original image (RGB color)



8-bit/gray scale/DAPI channel

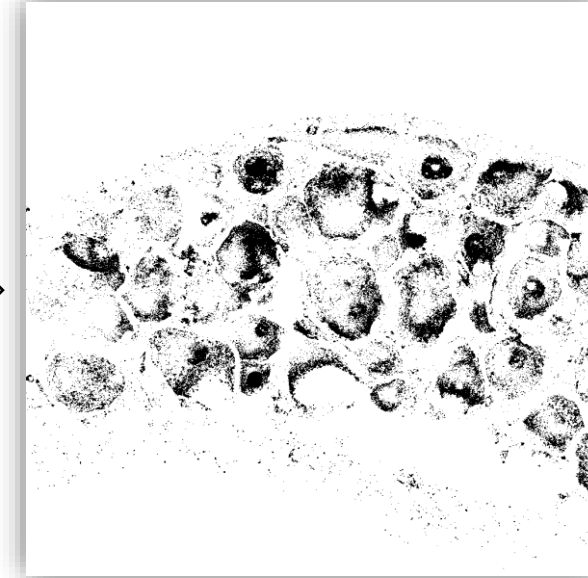


8-bit/gray scale/red channel

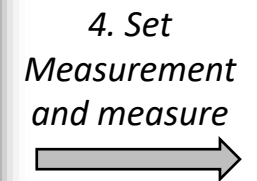


After Threshold\* but still in gray scale

\*Threshold is set based on non-primary images from the same experiments (day - should have been animals)



Binary image/red channel



## Image analysis strategy

*Pros: non-biased analysis of the signal intensity of VEGF R1 in the whole DRG*

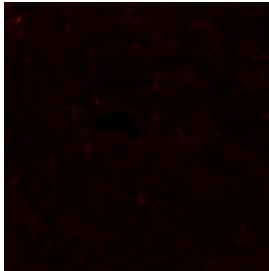
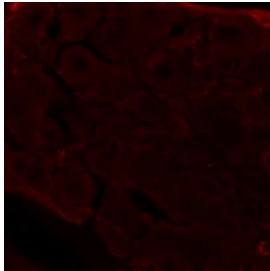
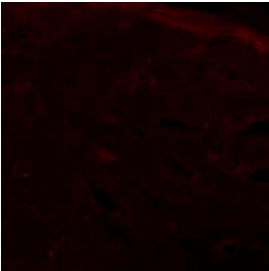
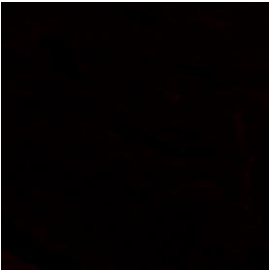
*Cons: no differentiation between expression in different cell types (neuronal vs glia) or subcellular locations (cytosol vs nuclei)*

Saline instilled

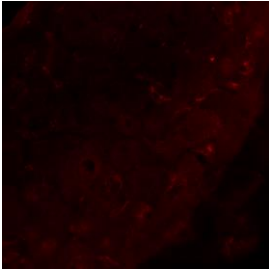
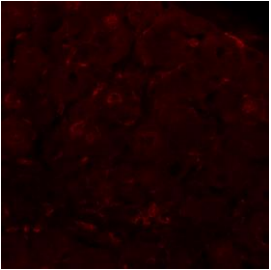
VEGF<sub>165</sub> instilled

No primary

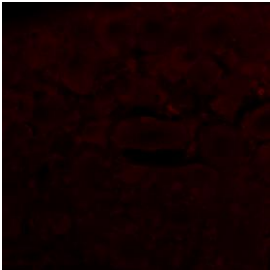
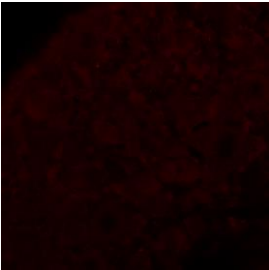
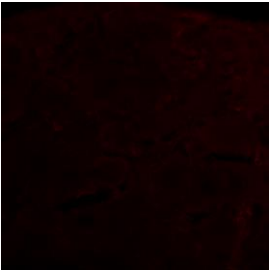
RG-F-R2378



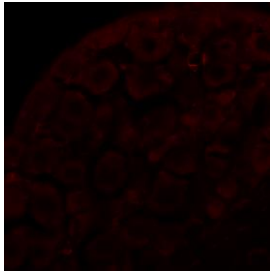
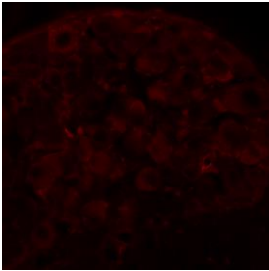
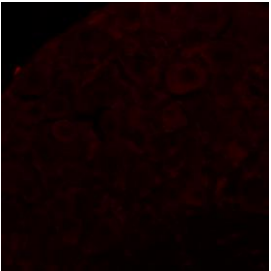
RG-M-R0162



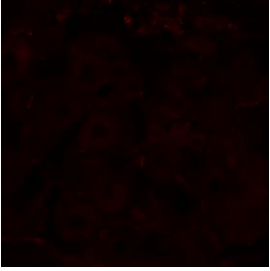
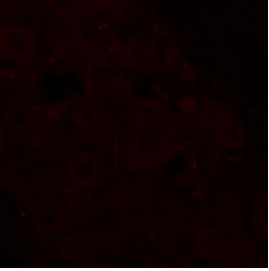
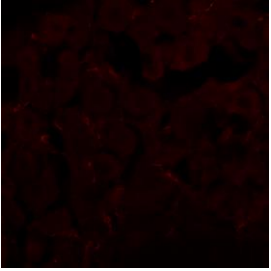
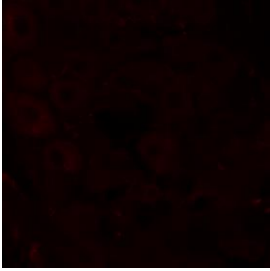
TG-F-R2280



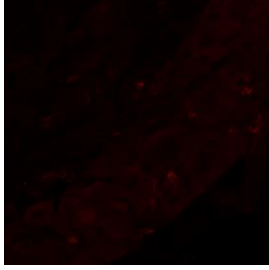
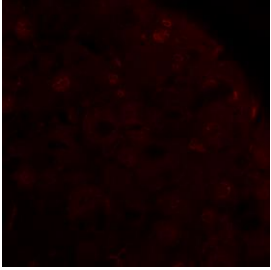
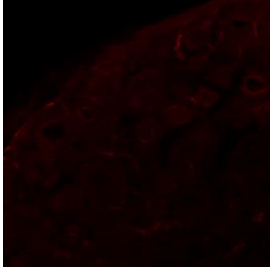
TG-M-R2273



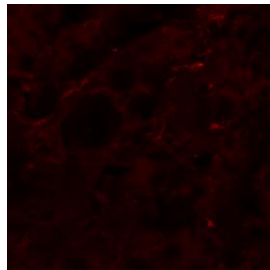
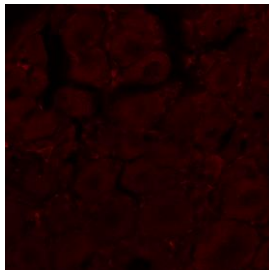
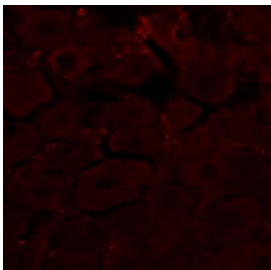
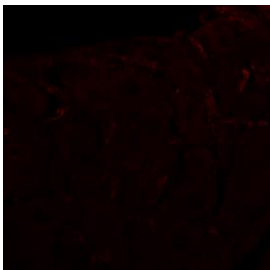
RG-F-R2377



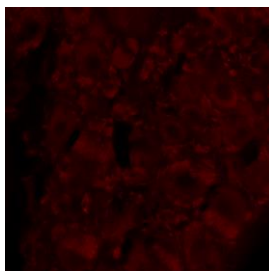
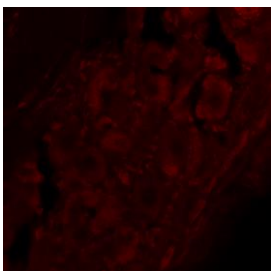
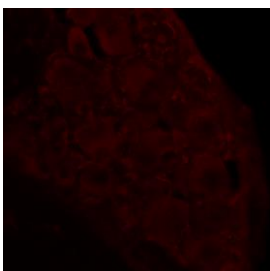
RG-M-R0161



TG-F-R2274



TG-M-R2277



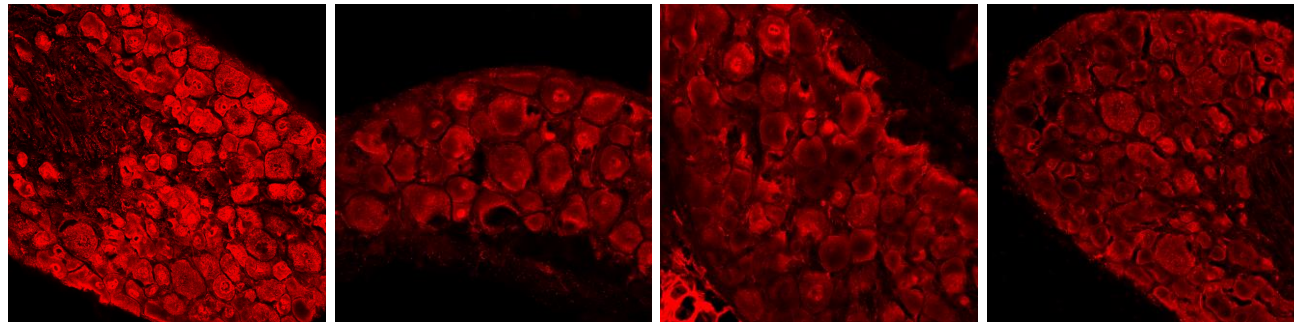
VEGF R1  
Staining day 1



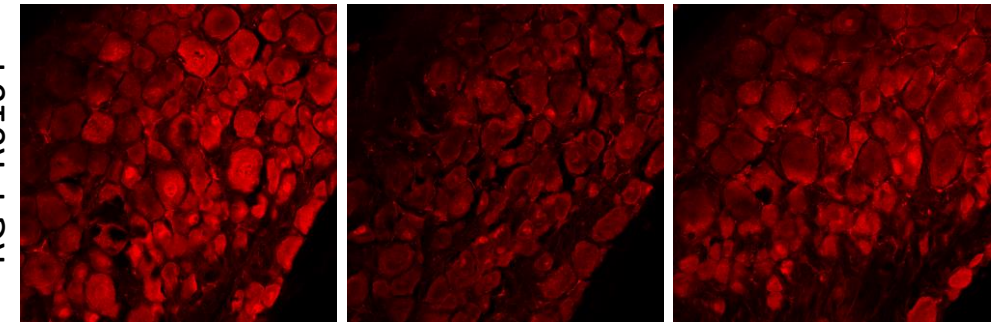
Saline instilled

VEGF<sub>165</sub> instilled

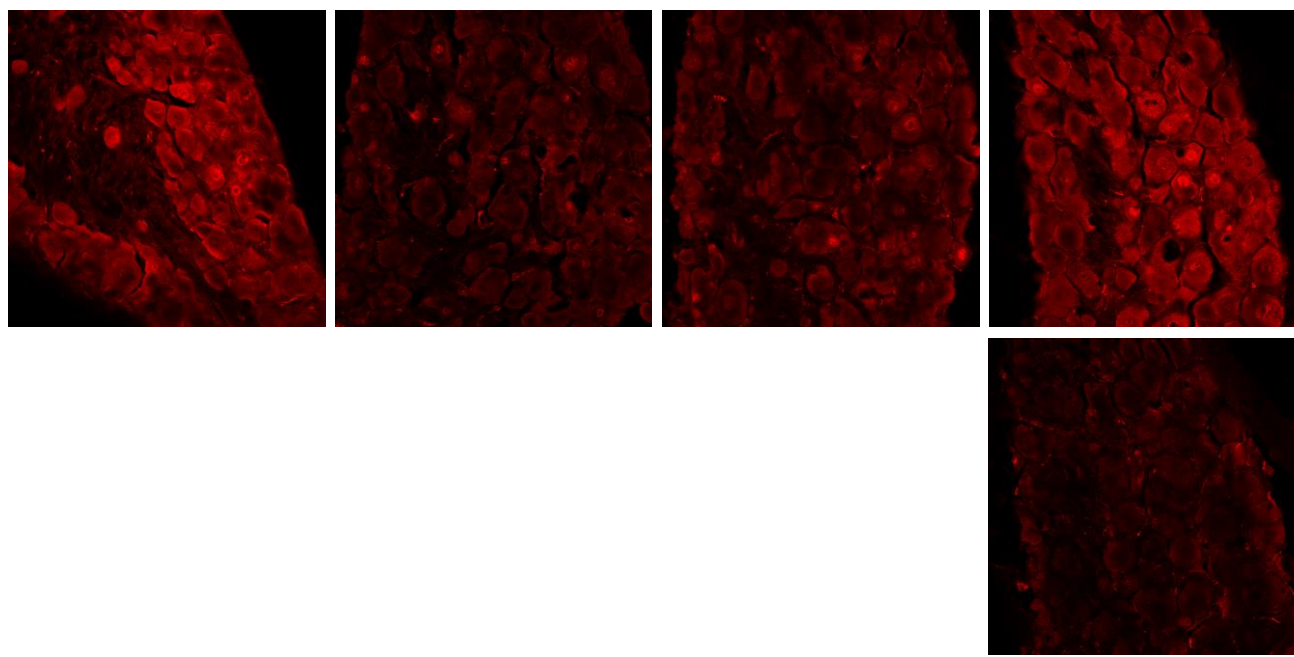
RG-F-R0165



RG-F-R0164



RG-M-R0163

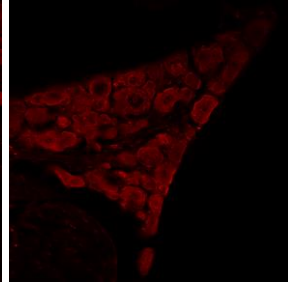
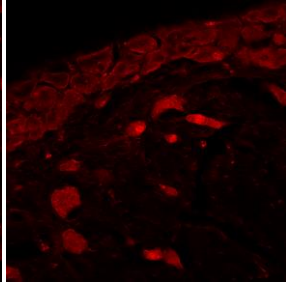
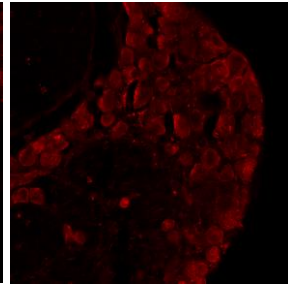
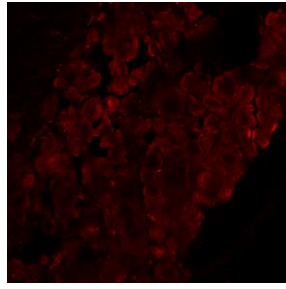
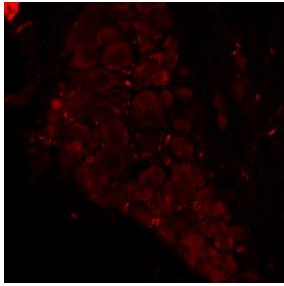


VEGF R1  
Staining day 2

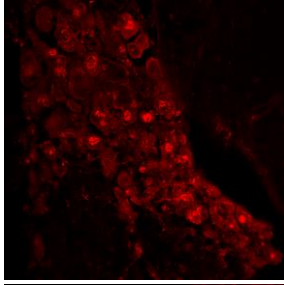
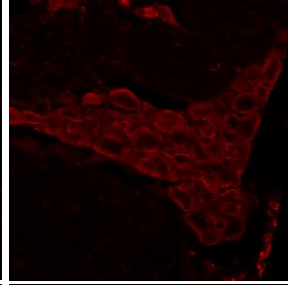
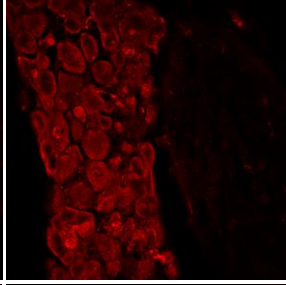
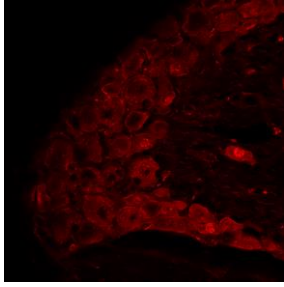
VEGF R1  
Staining day 3

Saline instilled

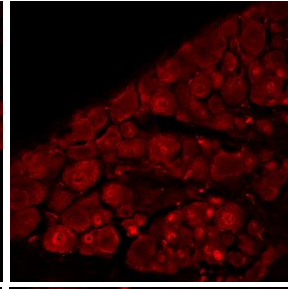
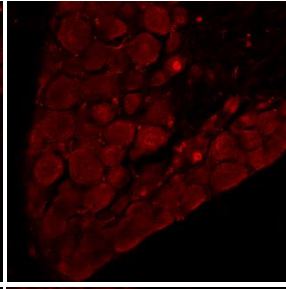
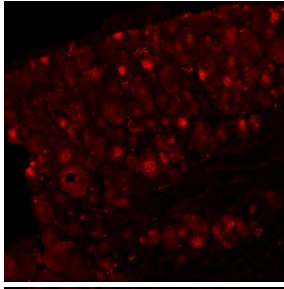
CG-M-R2375



CG-M-R2575

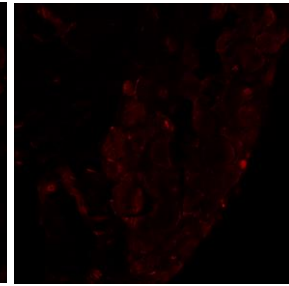
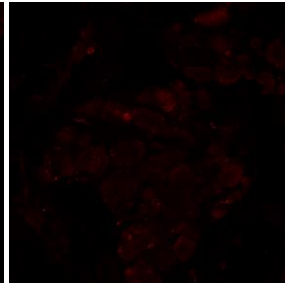
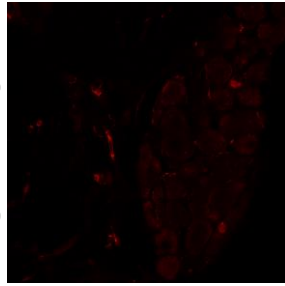


CG-F-R7079

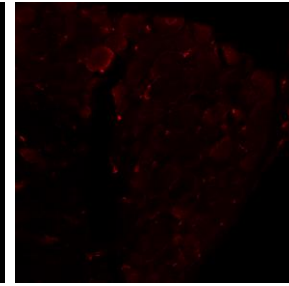
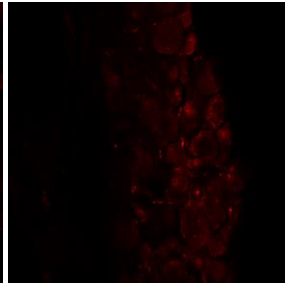
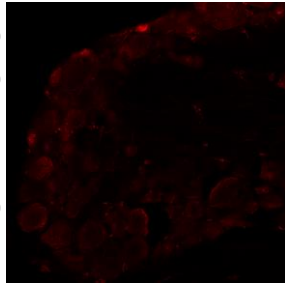


VEGF<sub>165</sub> instilled

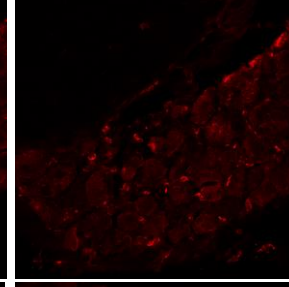
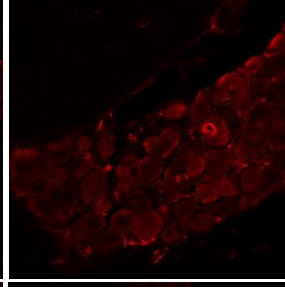
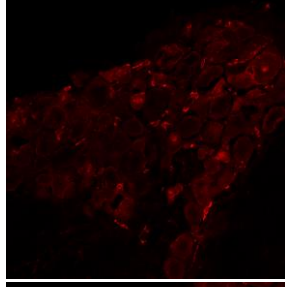
RG-M-R2374



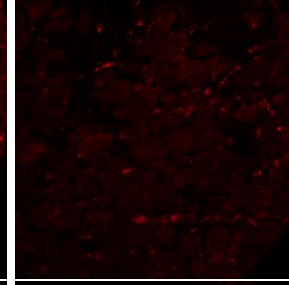
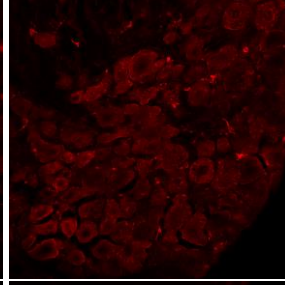
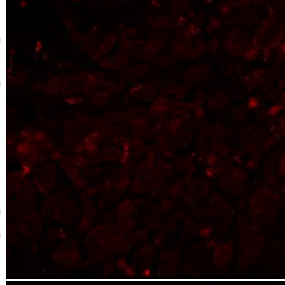
RG-M-R2373



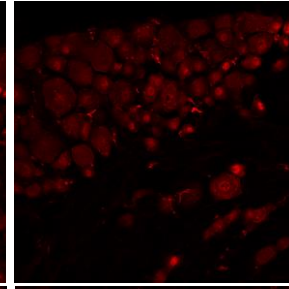
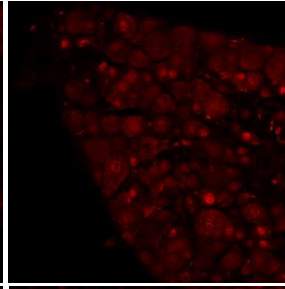
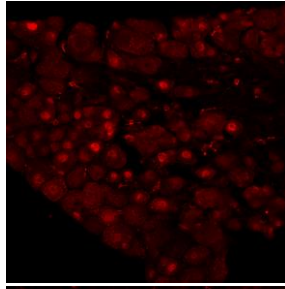
RG-F-R2376



CG-M-R2573



CG-F-R7078

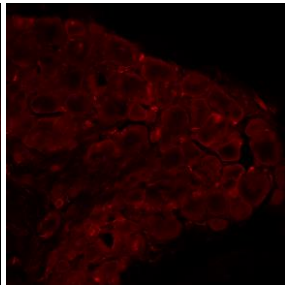
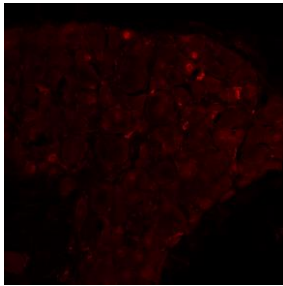
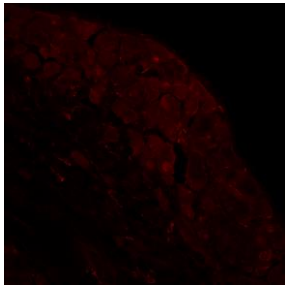




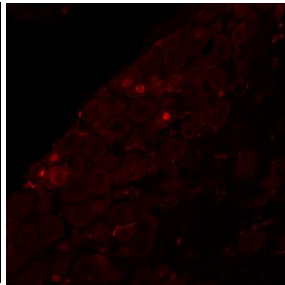
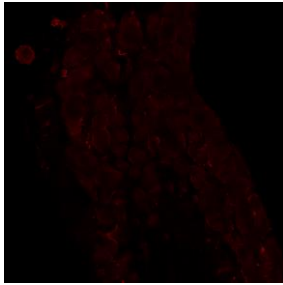
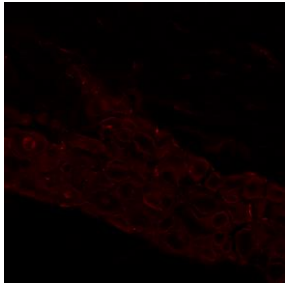
VEGF R1  
Staining day 4 CGs

Saline instilled

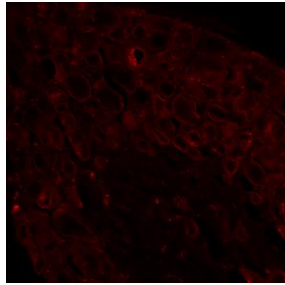
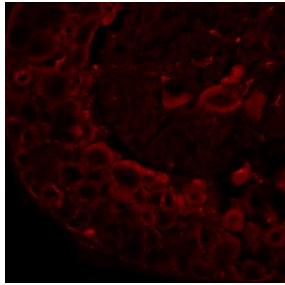
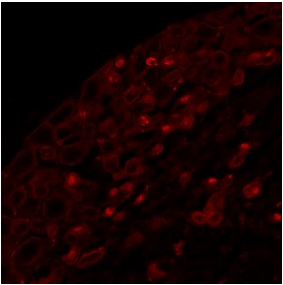
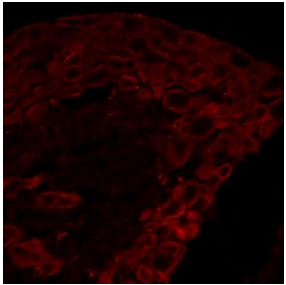
CG-M-R2118



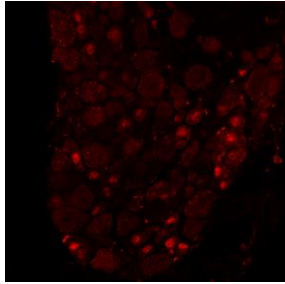
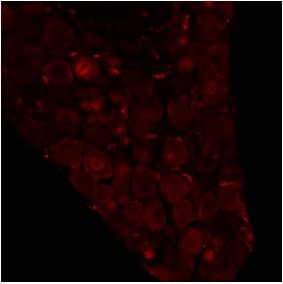
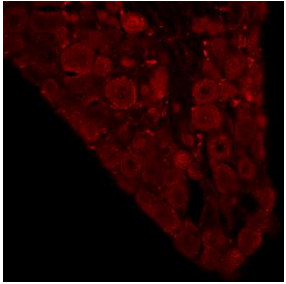
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CG-F-R2580

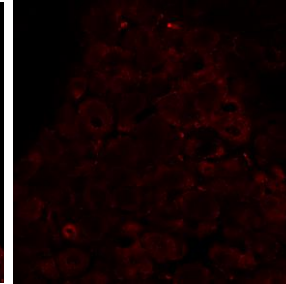
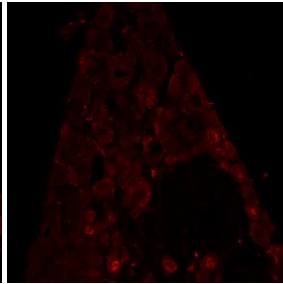
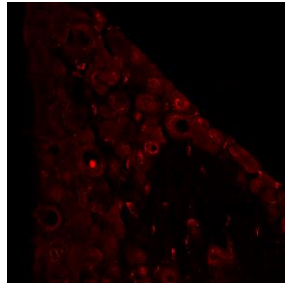


CG-F-R2124

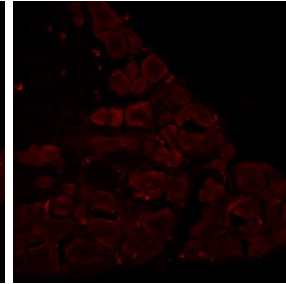
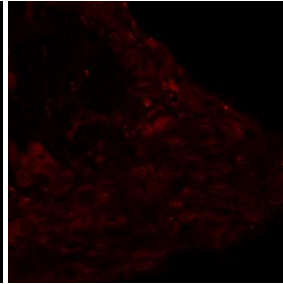
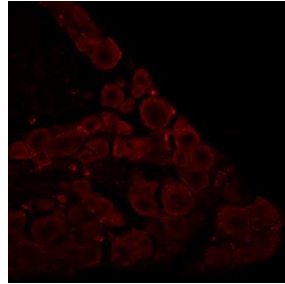


VEGF<sub>165</sub> instilled

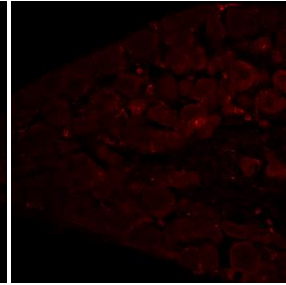
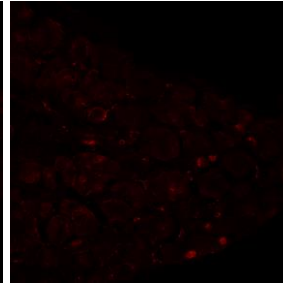
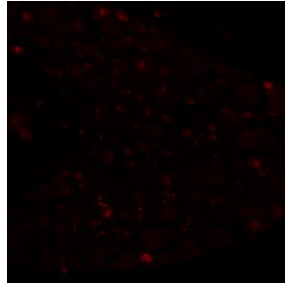
CG-M-R2115



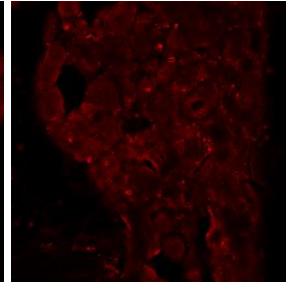
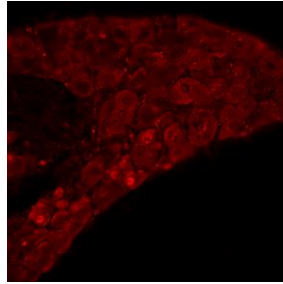
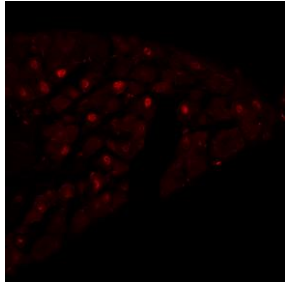
CG-F-R2579



CG-F-R2578



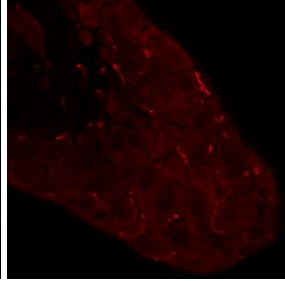
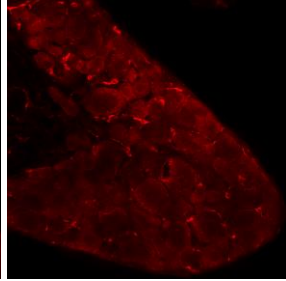
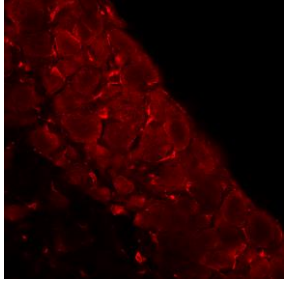
CG-F-R2577



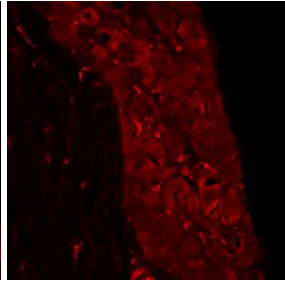
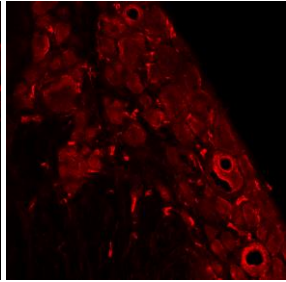
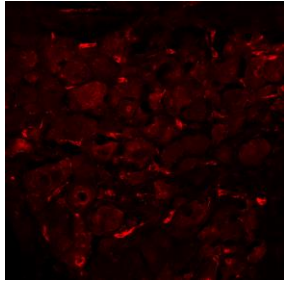


Saline instilled

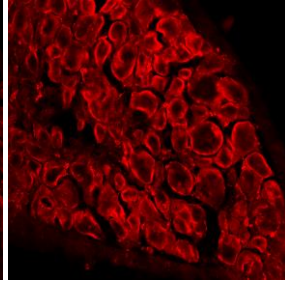
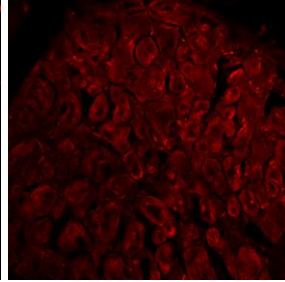
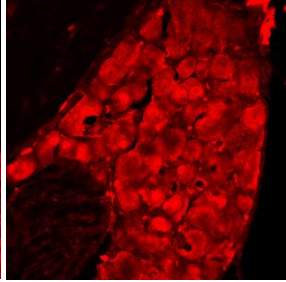
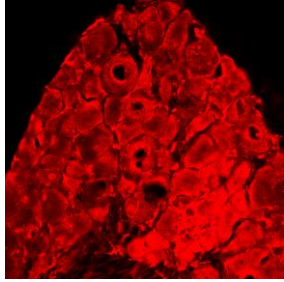
TG-M-R2272



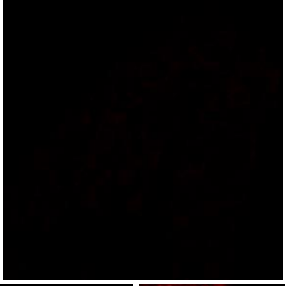
TG-M-R2497



TG-M-R2500



No primary  
5% laser



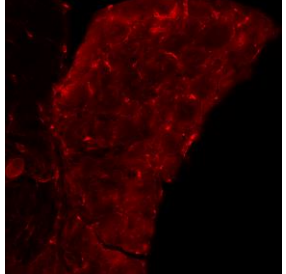
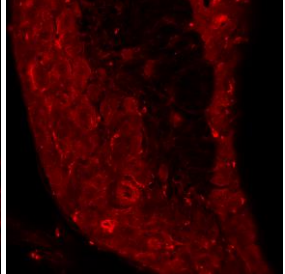
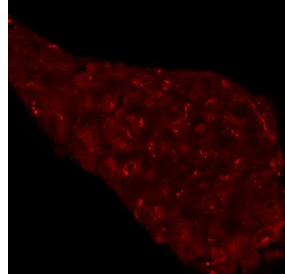
No primary  
20% laser



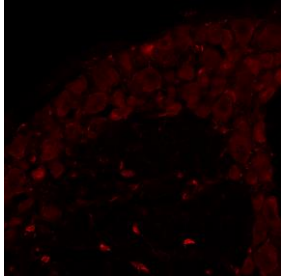
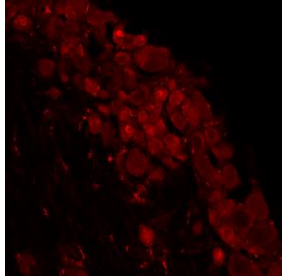
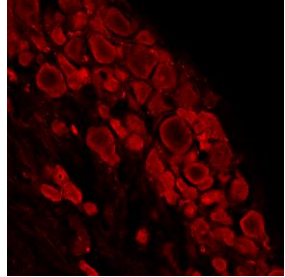
VEGF R1  
Staining day 4 TGs

VEGF<sub>165</sub> instilled

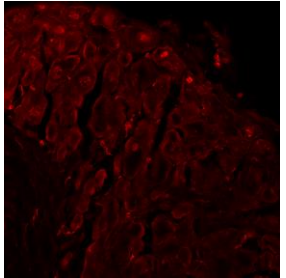
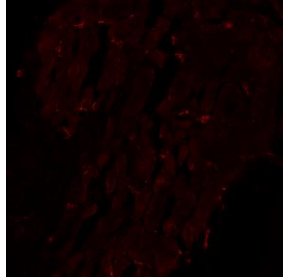
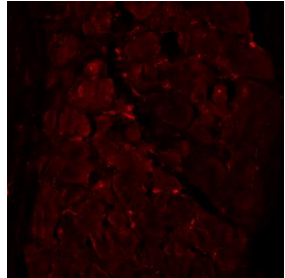
TG-M-R2496



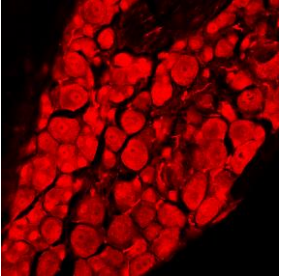
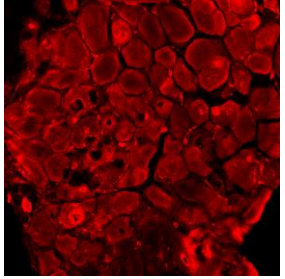
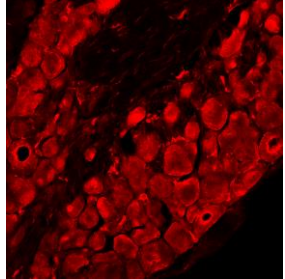
TG-M-R2278



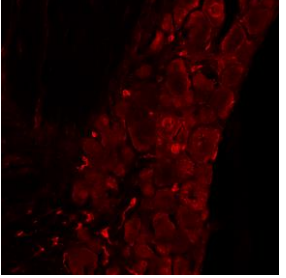
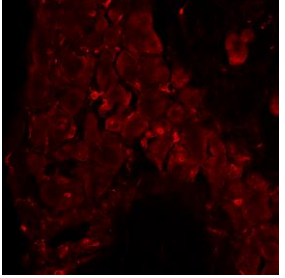
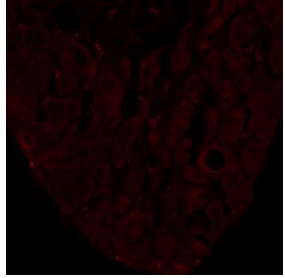
TG-M-R2276

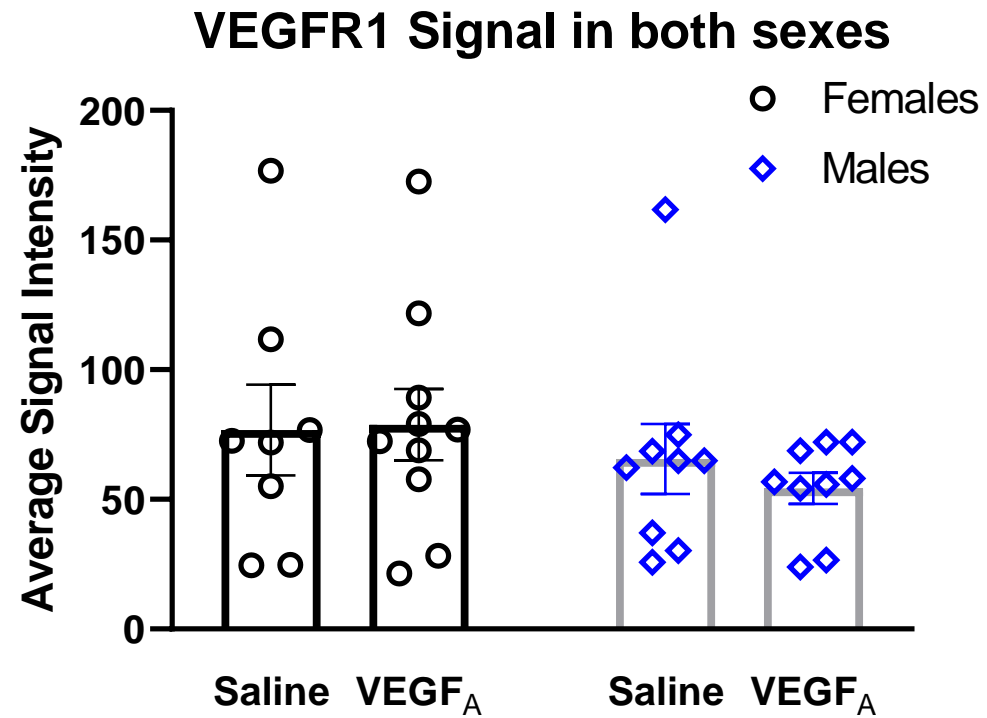


TG-F-R2499

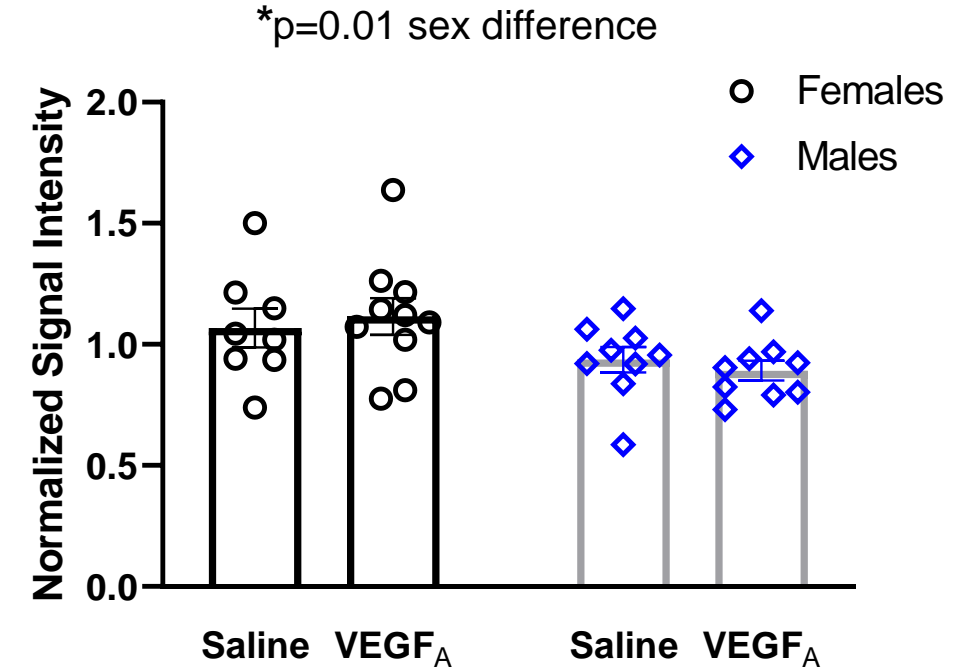


TG-F-R2275





### \*Normalized VEGFR1 Signal in both sexes



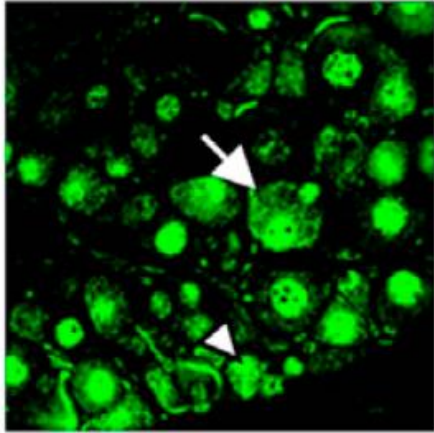
|             |    |   |    |
|-------------|----|---|----|
| Total count | 36 | M | F  |
| Saline      | 17 | 9 | 8  |
| VEGF165     | 19 | 9 | 10 |

\* Normalized to the average intensity of the saline-instilled tissue from the same staining day.

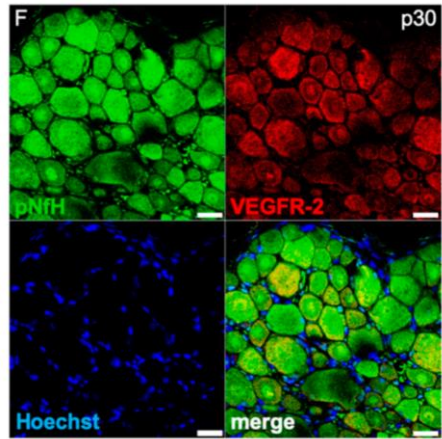


Two schools of thoughts:

## A anti-VEGFR2



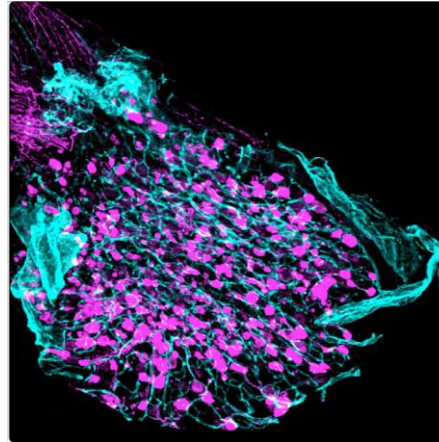
## B



VEGFR-2 is robustly expressed by endothelial cells throughout the extensive DRG capillary network, but not found in sensory neurons or other nonendothelial cells of the DRG.

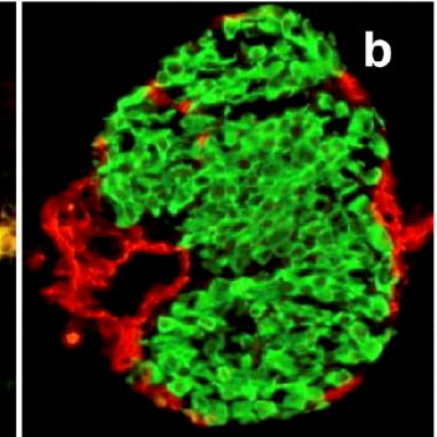
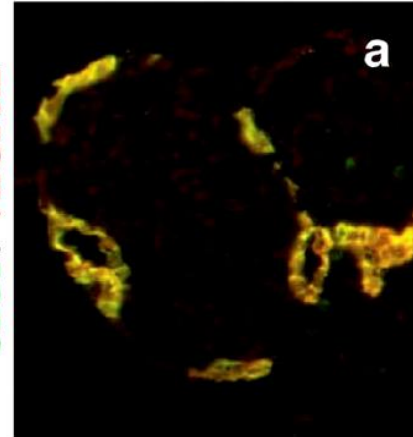
## C

CD31+ blood vessels



## D

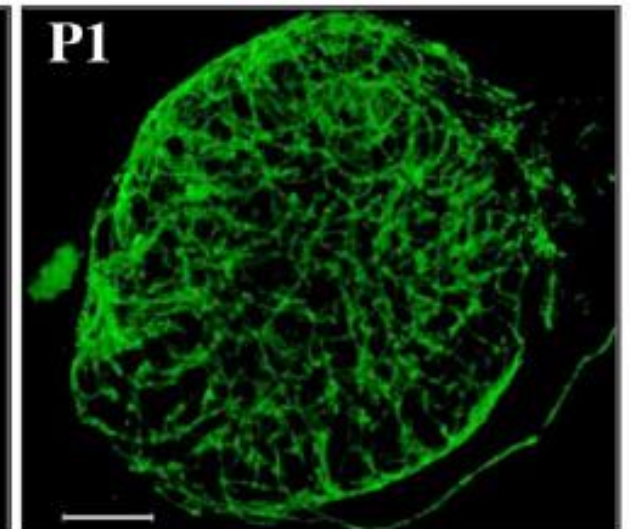
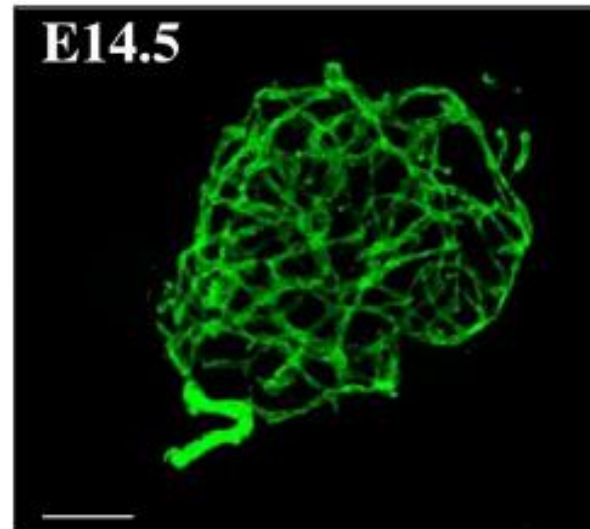
CD31 / VEGFR2

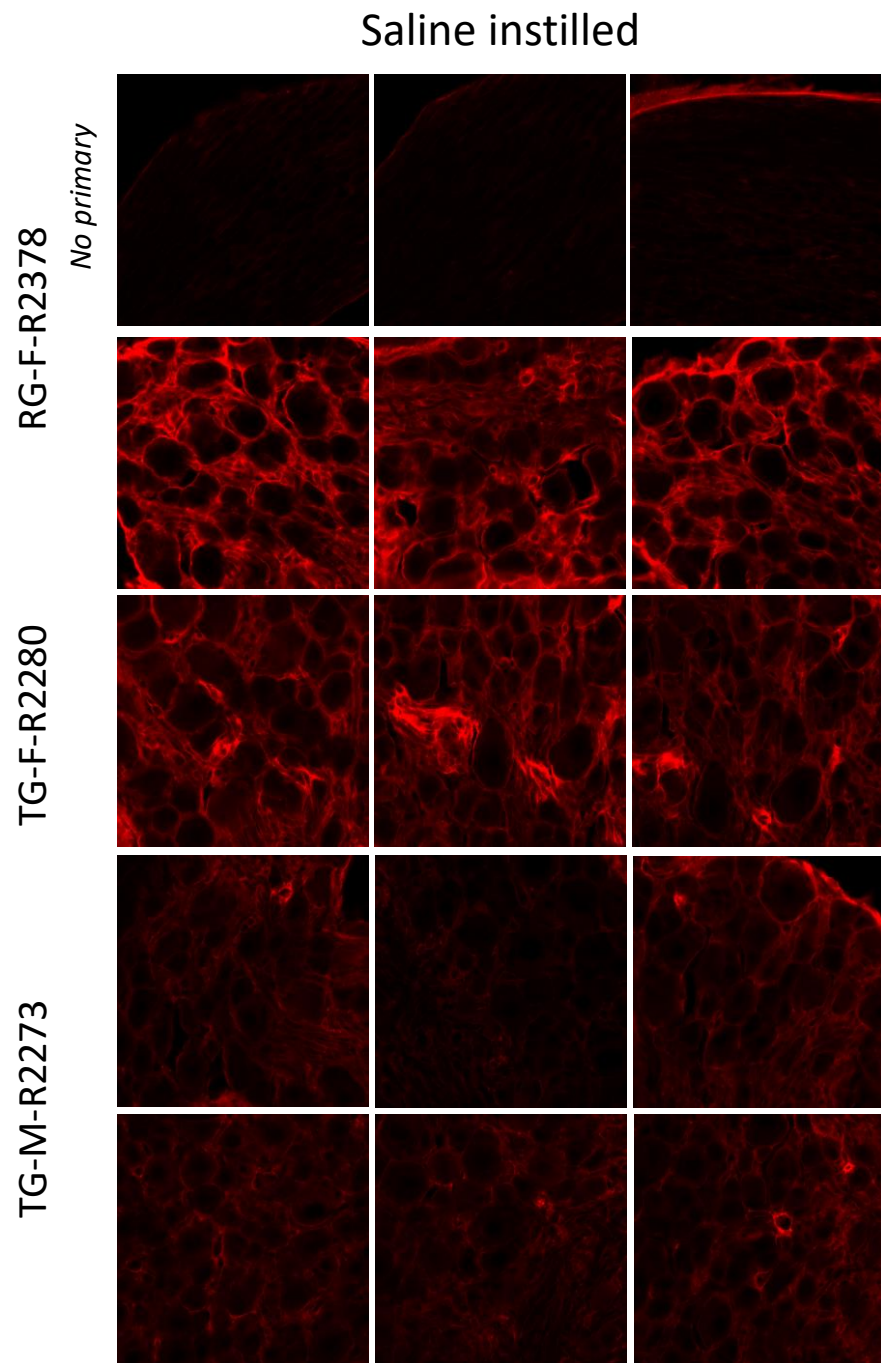


$\beta$ III tubulin / VEGFR2

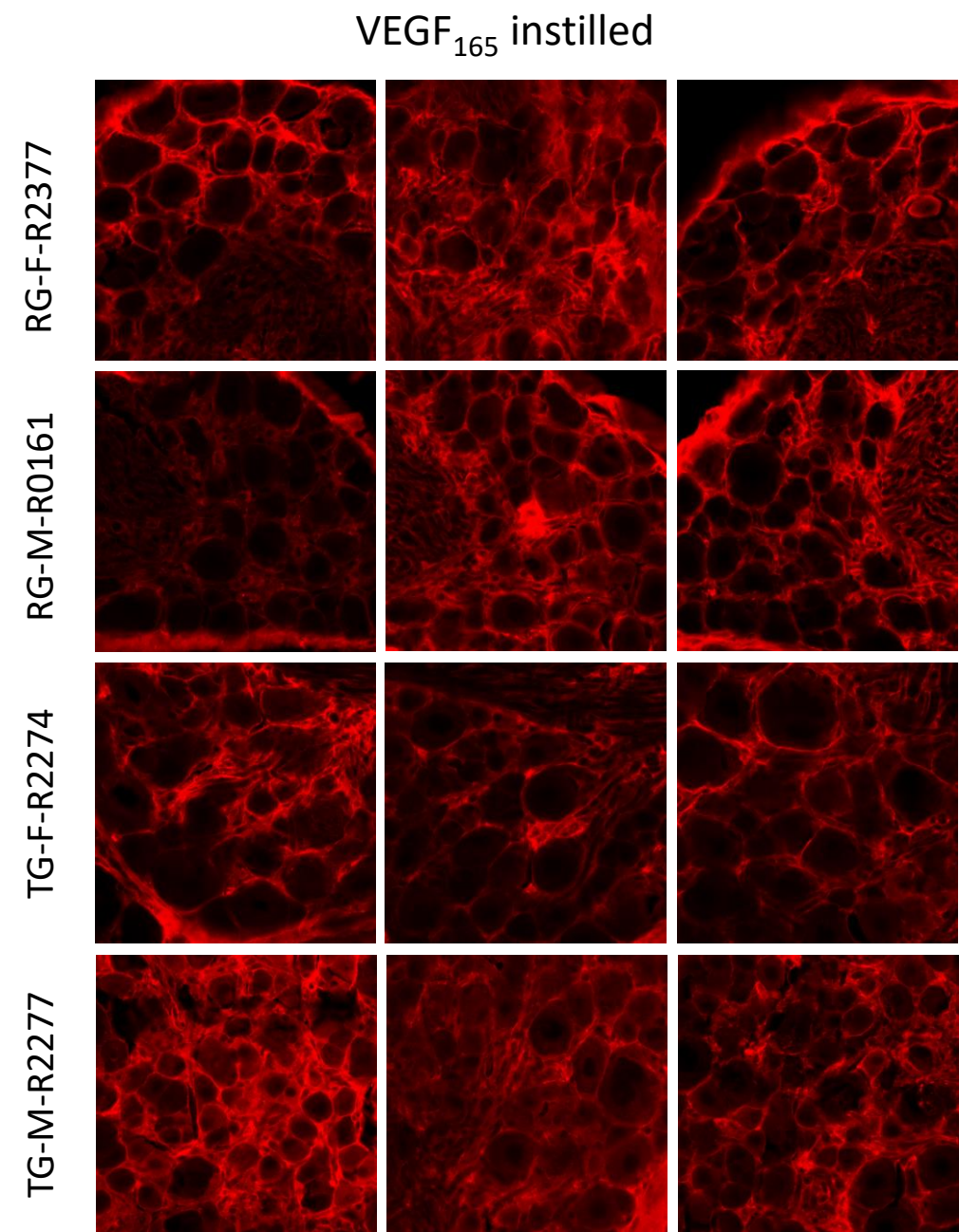
## D Freshly dissected

CD31





VEGF R2  
Staining day 1

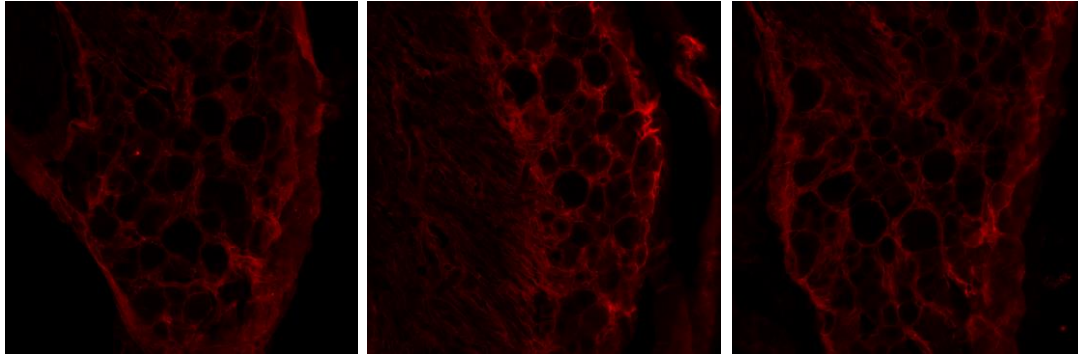




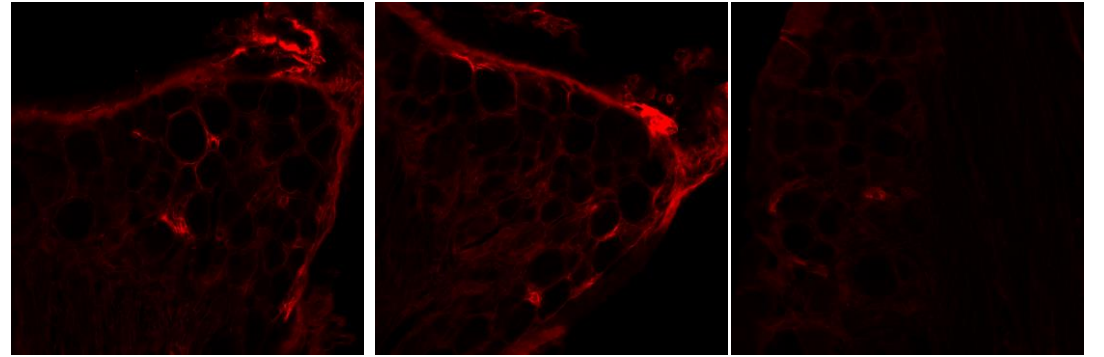
Saline instilled

VEGF<sub>165</sub> instilled

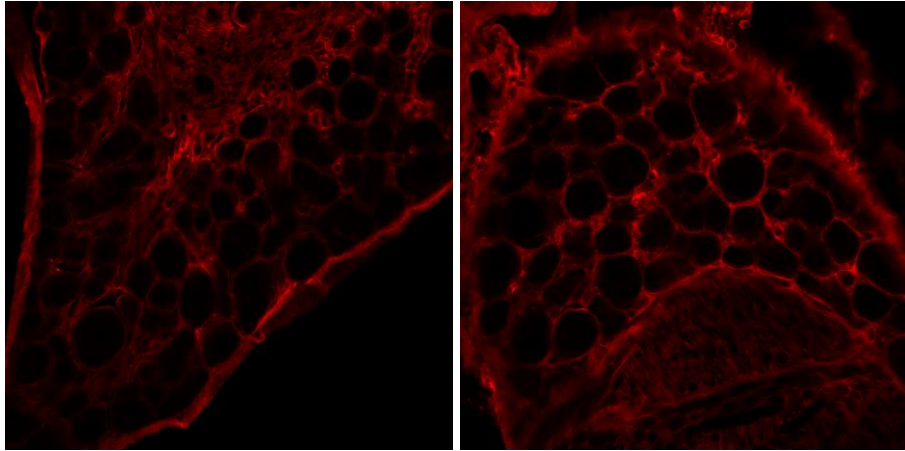
RG-F-R0165



RG-F-R0164



RG-M-R0163



VEGF R2  
Staining day 2

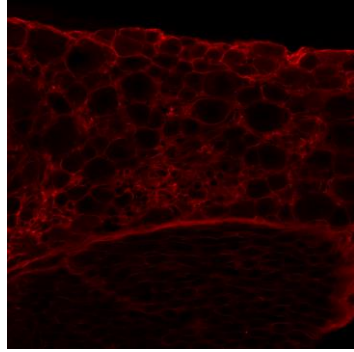
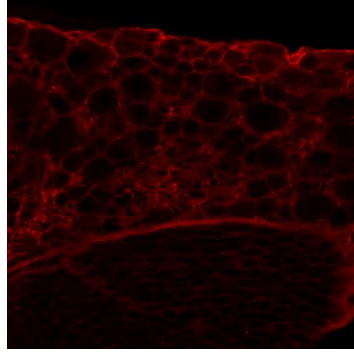
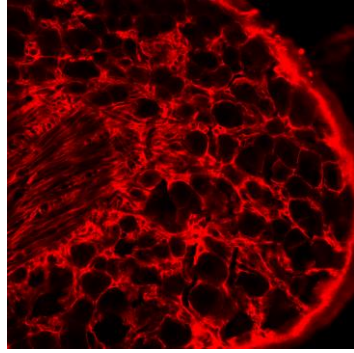
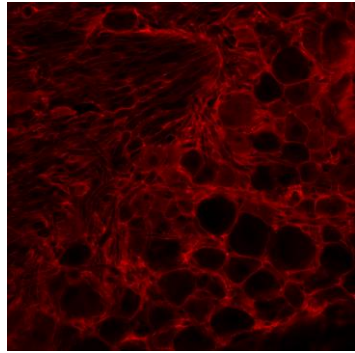
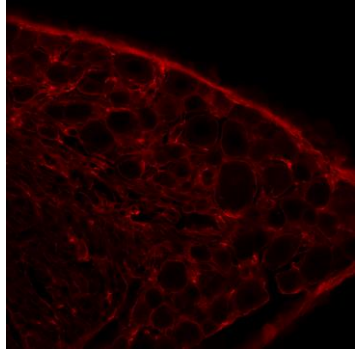
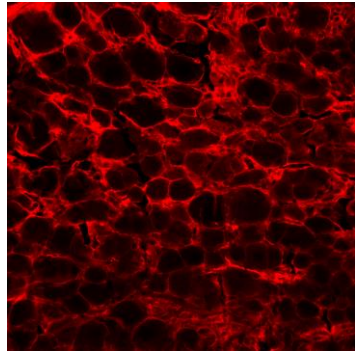
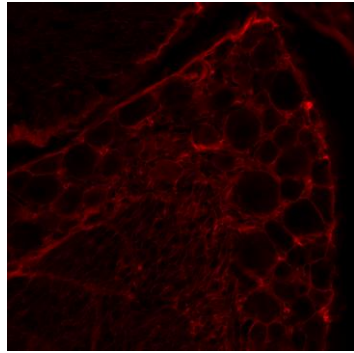
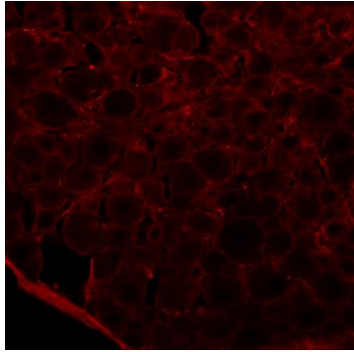
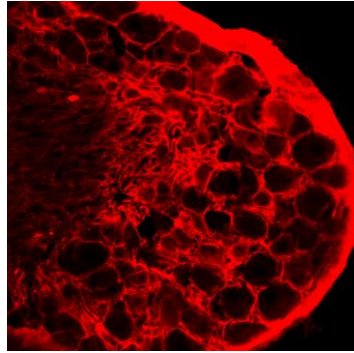
VEGF R2  
Staining day 3

Saline instilled

CG-M-R2375

CG-M-R2575

CG-F-R7079



VEGF<sub>165</sub> instilled

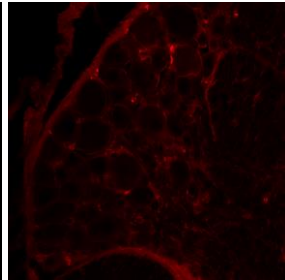
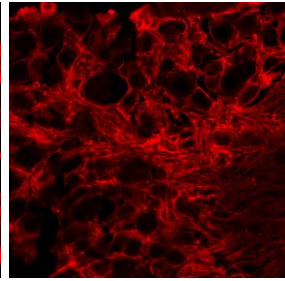
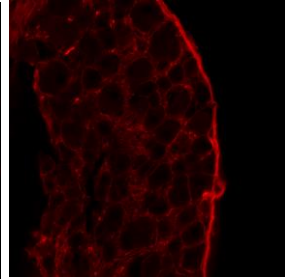
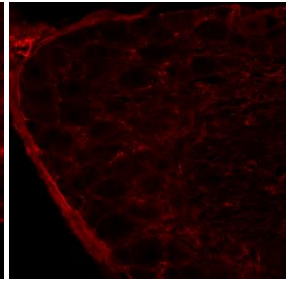
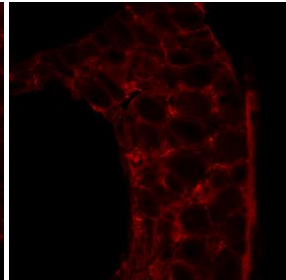
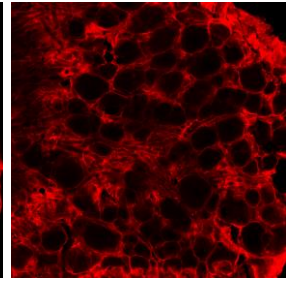
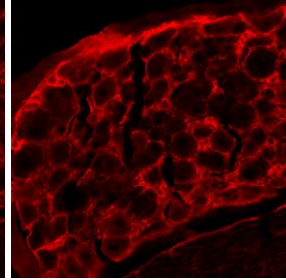
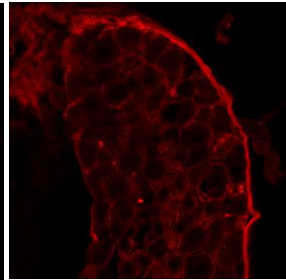
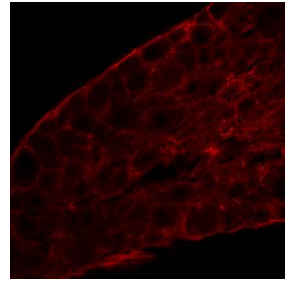
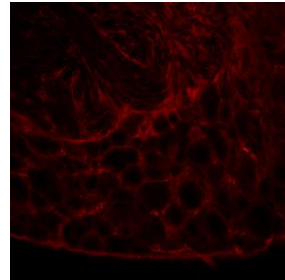
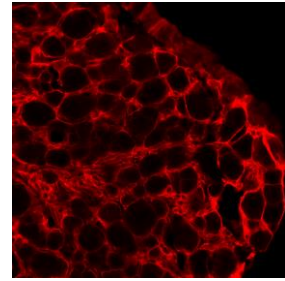
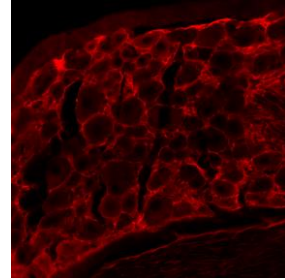
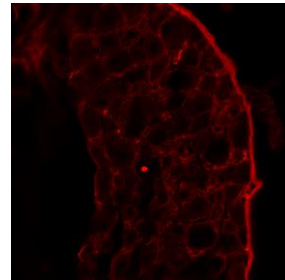
RG-M-R2374

RG-M-R2373

RG-F-R2376

CG-M-R2573

CG-F-R7078





VEGF R2  
Staining day 4 CGs

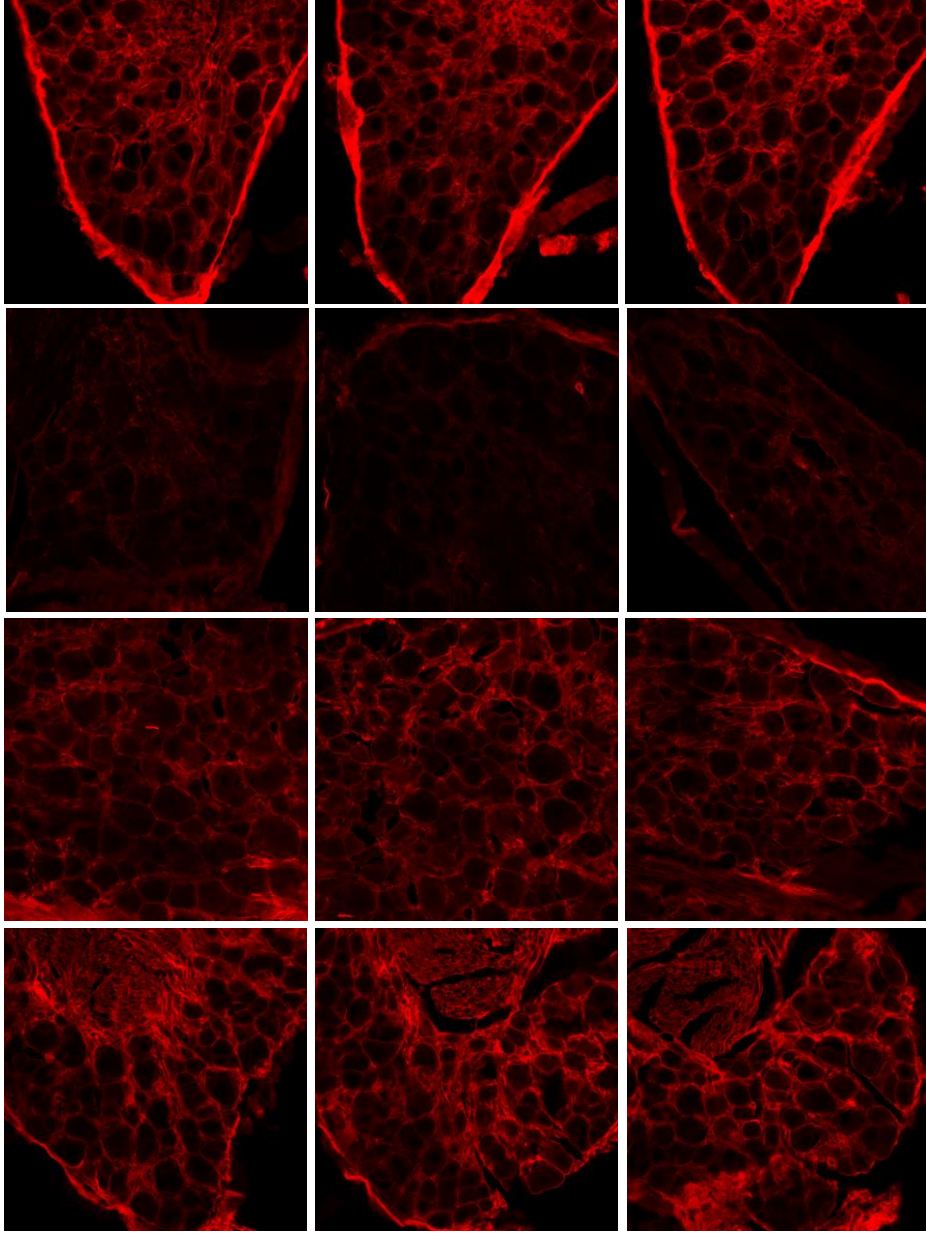
Saline instilled

CG-F-R2124

CG-F-R2580

CG-M-R2117

CG-M-R2118



VEGF<sub>165</sub> instilled

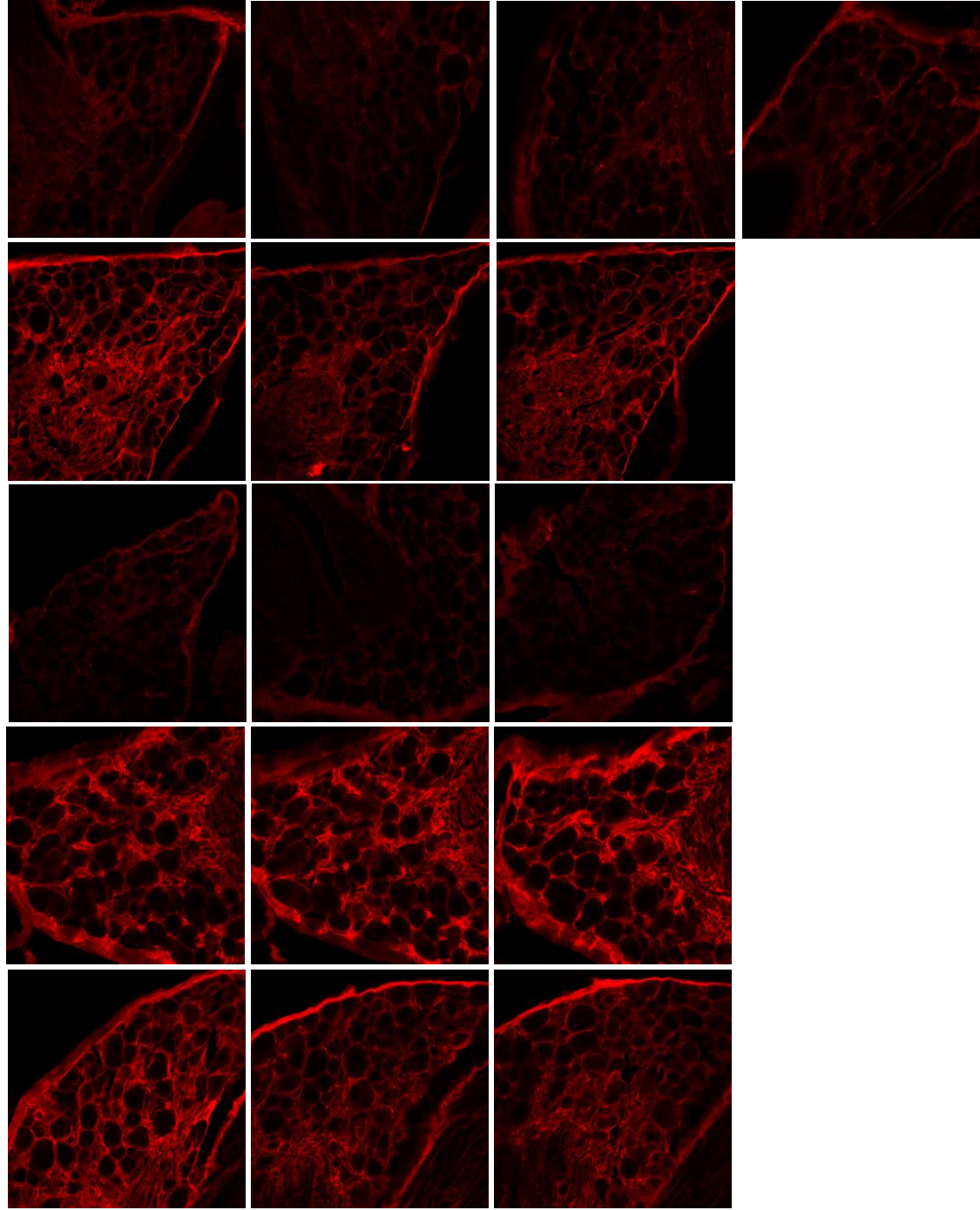
CG-M-R2116

CG-M-R2115

CG-F-R2579

CG-F-R2578

CG-F-R2577

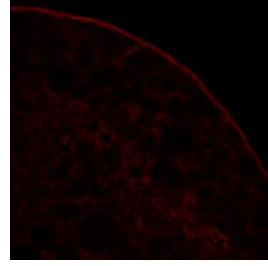


No primary



Saline instilled

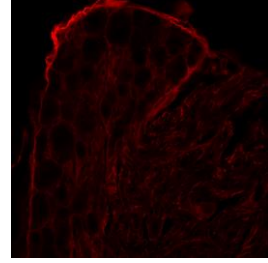
TG-M-R2272



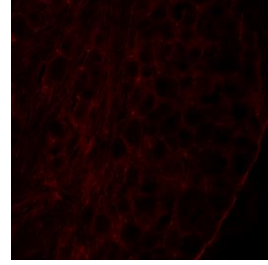
No primary

VEGF R2  
Staining day 5 TGs

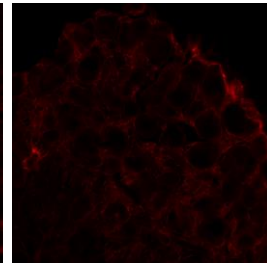
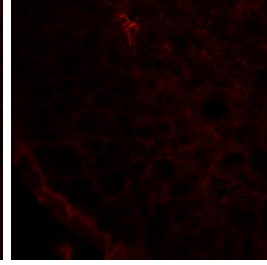
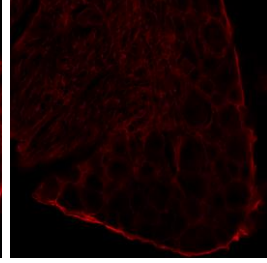
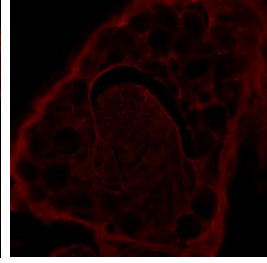
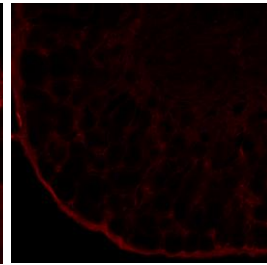
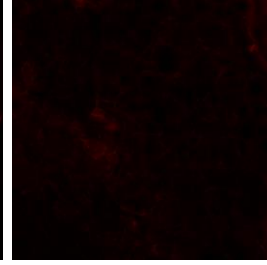
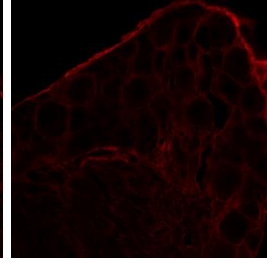
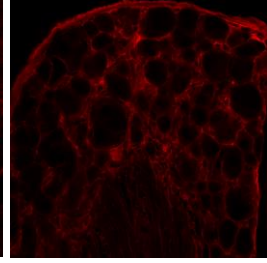
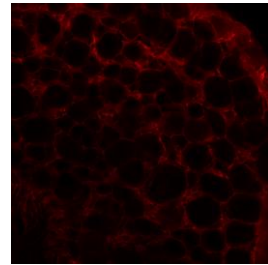
TG-F-R2279



TG-M-R2497

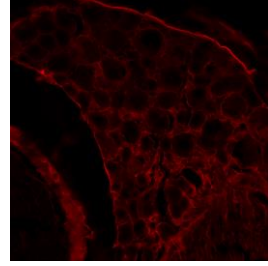


TG-M-R2500

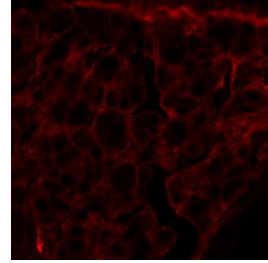


VEGF<sub>165</sub> instilled

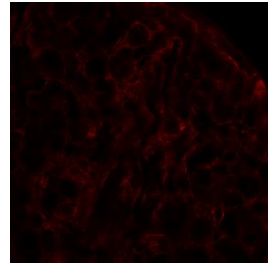
TG-F-R2275



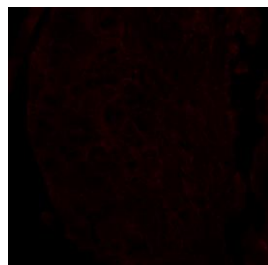
TG-F-R2499



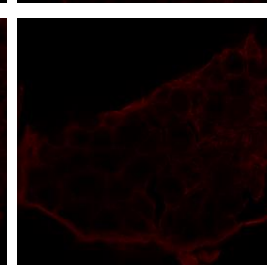
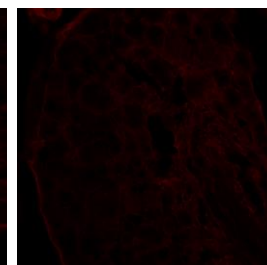
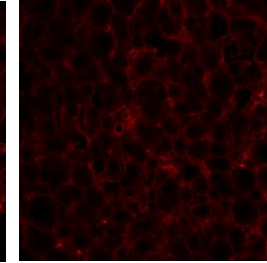
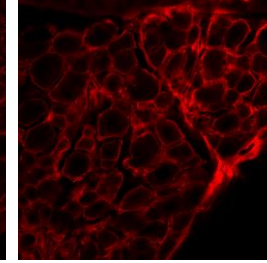
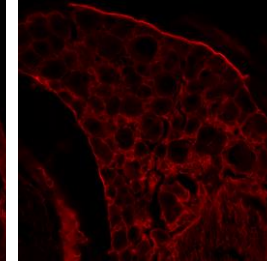
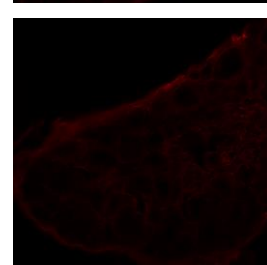
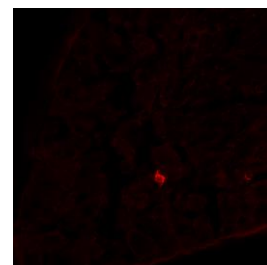
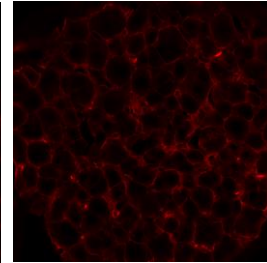
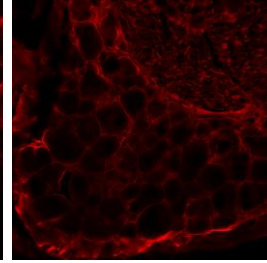
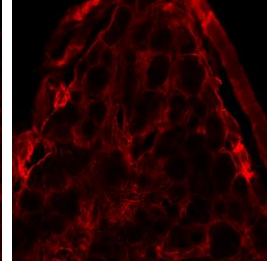
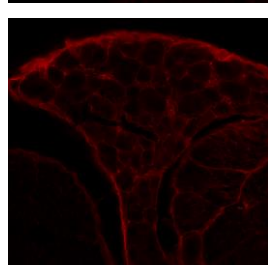
TG-M-R2276



TG-M-R2278

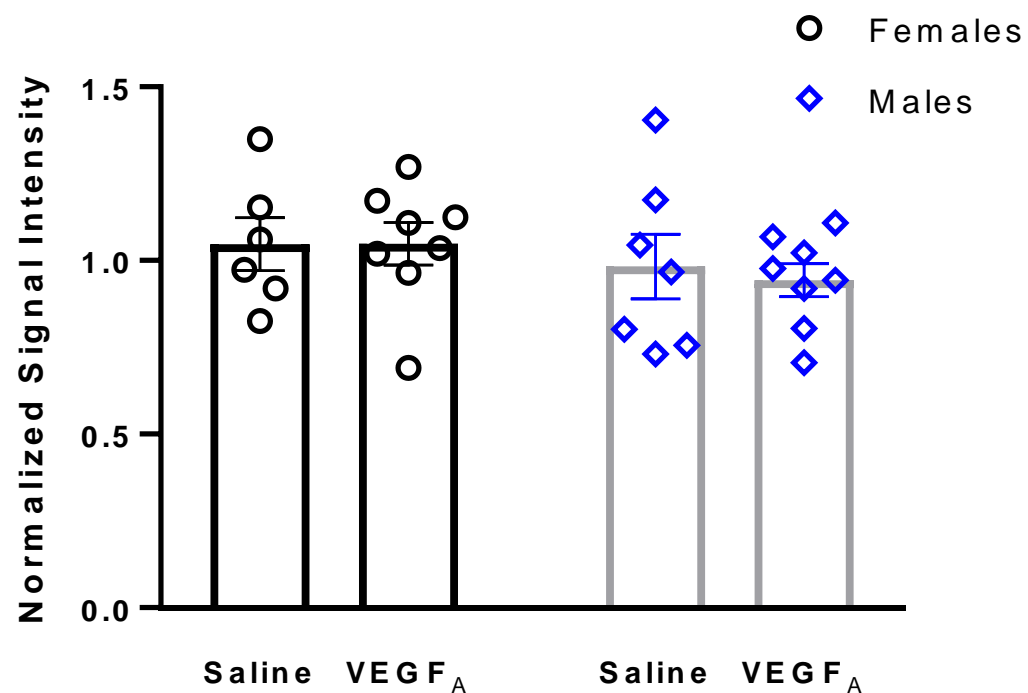


TG-M-R2496

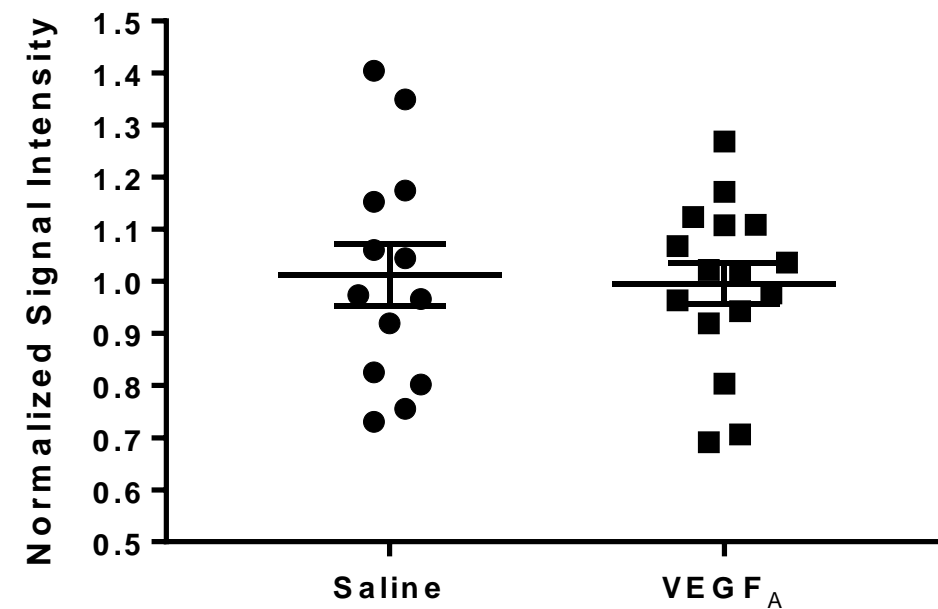




**Normalized VEGFR2 Signal in both sexes**

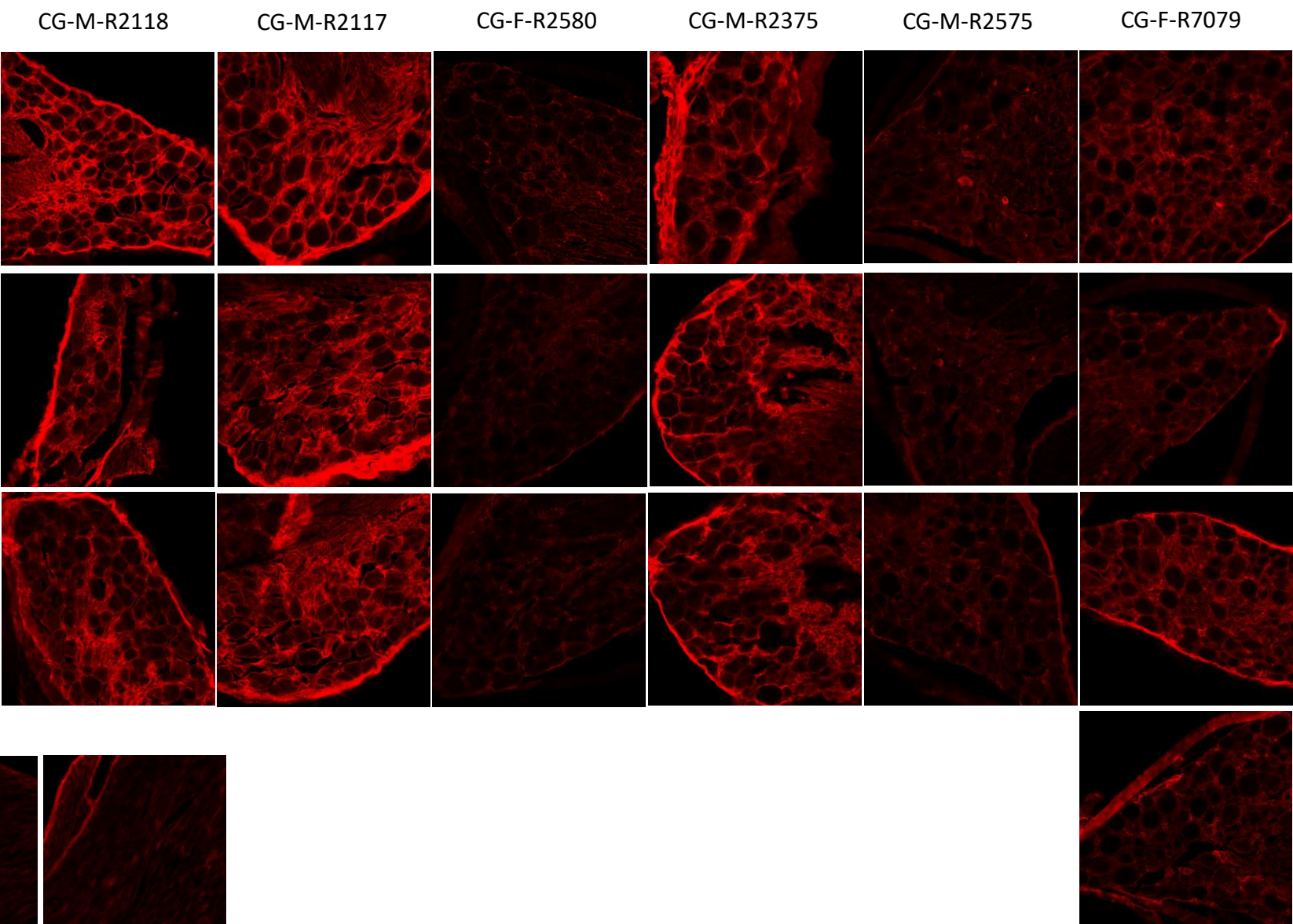


**Normalized VEGFR2 Signal; combined sexes**



|             |    |   |   |
|-------------|----|---|---|
| Total count | 31 | M | F |
| Saline      | 14 | 7 | 7 |
| VEGF165     | 17 | 9 | 8 |

TRPV1 Staining Saline Instillation - 1





RG-F-R0165

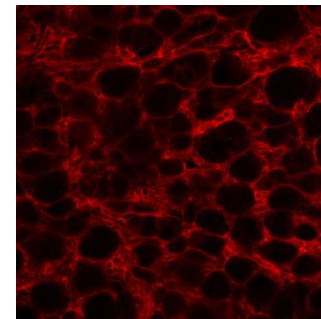
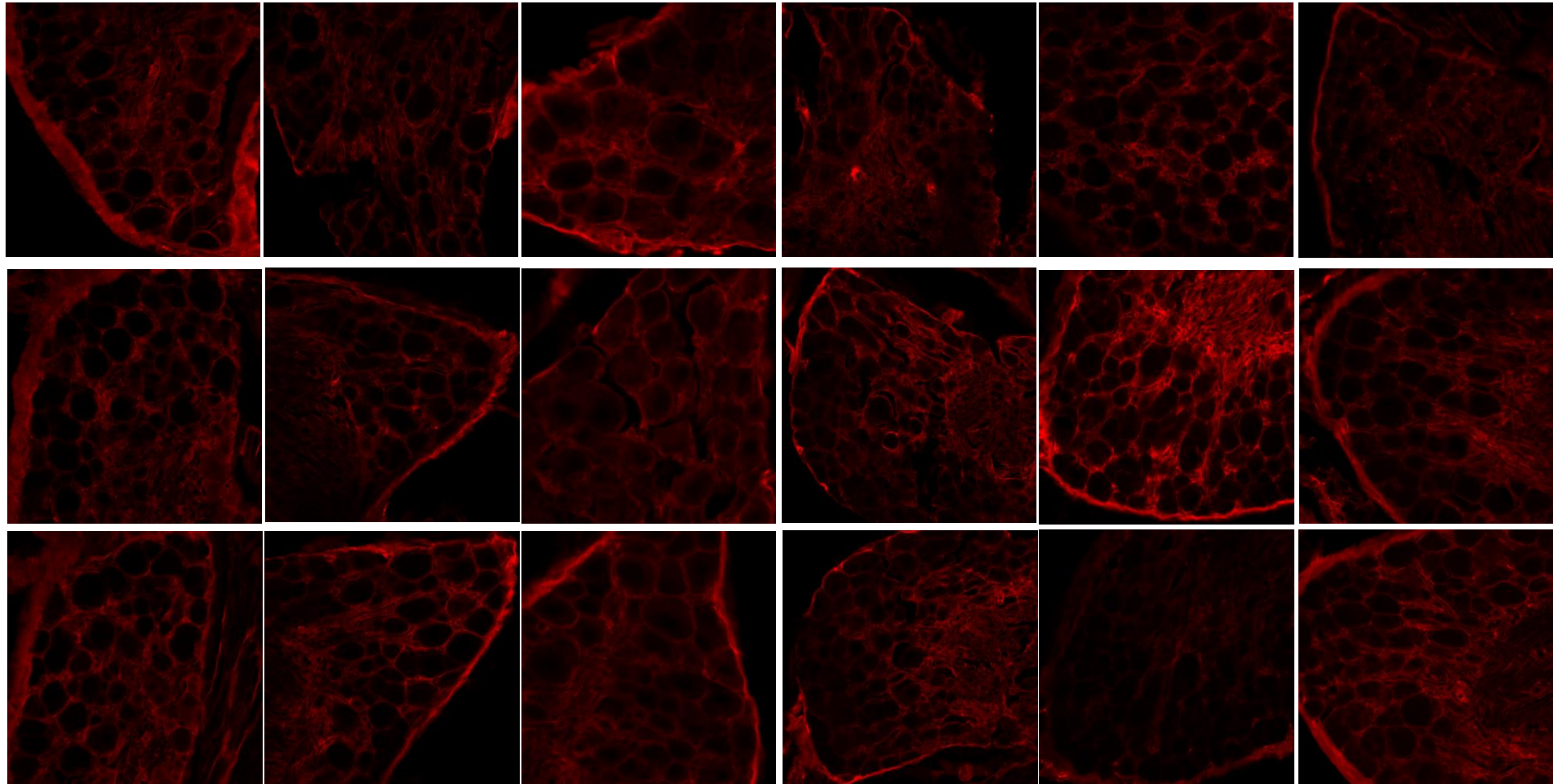
RG-M-R0163

TG-M-R2273

CG-F-R2279

CG-F-R2500

TG-M-R2272



TRPV1 Staining Saline Instillation - 2

CG-M-R2116

CG-M-R2115

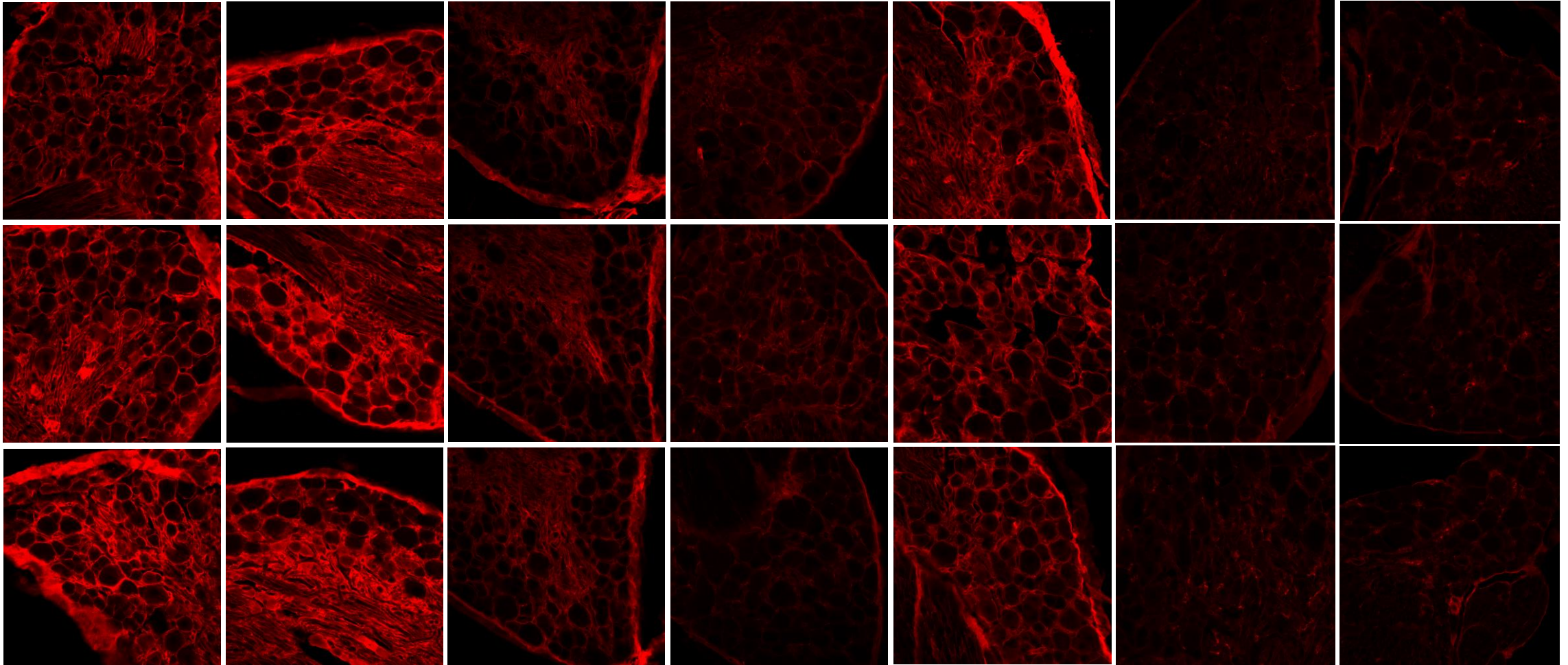
CG-F-R2579

CG-F-R2577

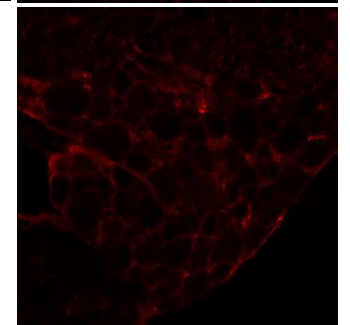
RG-F-R2376

CG-F-P7078

CG-M-R2573

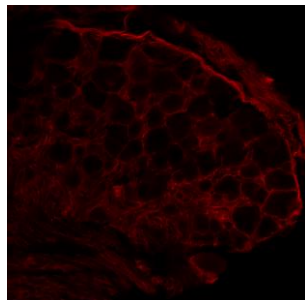


## TRPV1 Staining VEGF<sub>A</sub> Instillation - 1

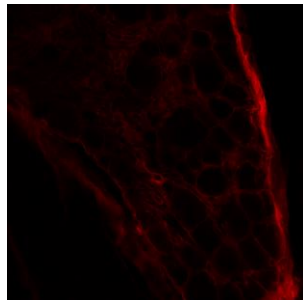




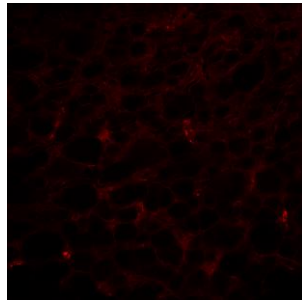
RG-M-R2373



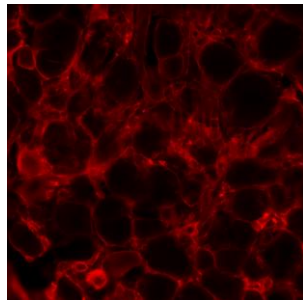
RG-F-R2376



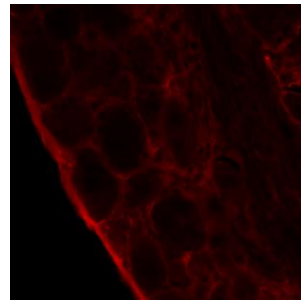
RG-F-R0164



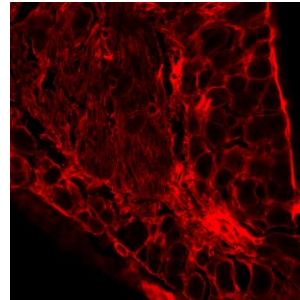
RG-F-R2377



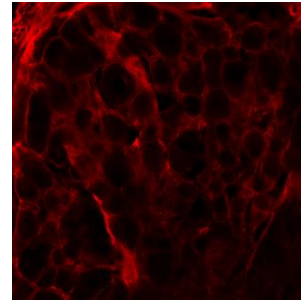
TG-M-R2277



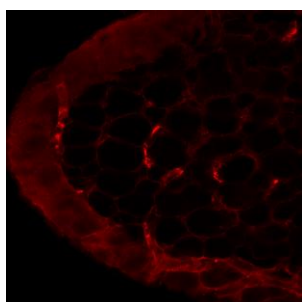
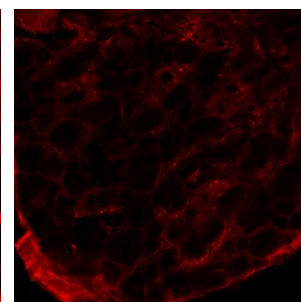
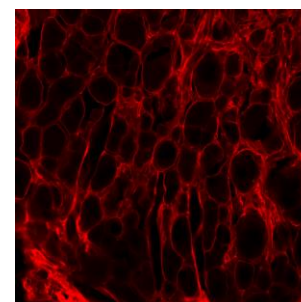
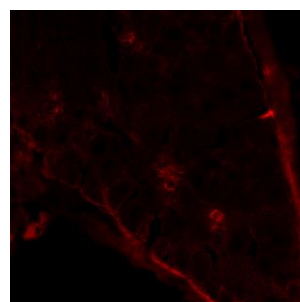
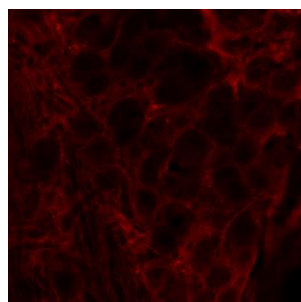
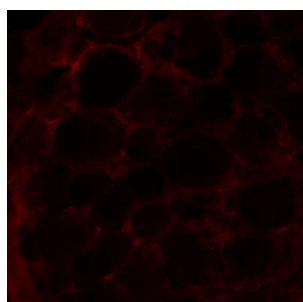
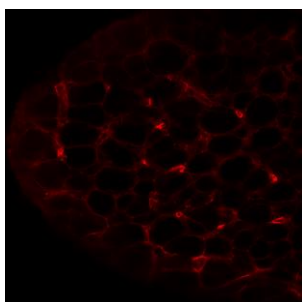
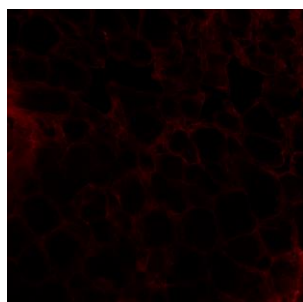
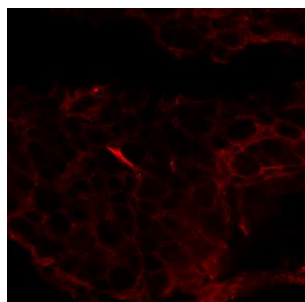
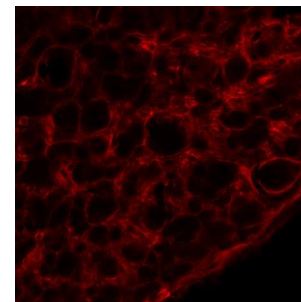
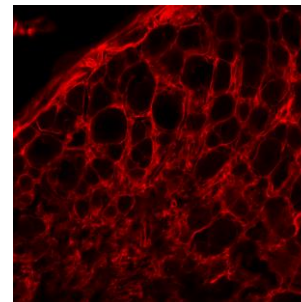
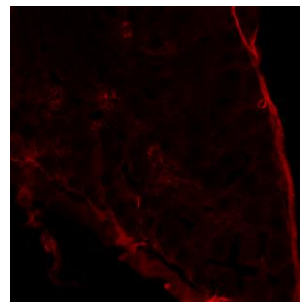
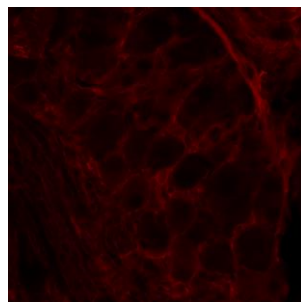
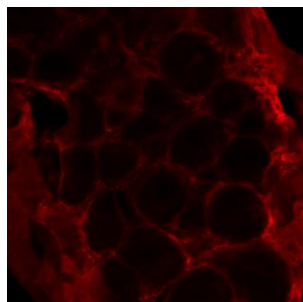
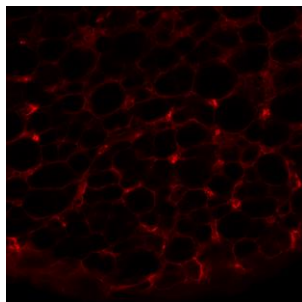
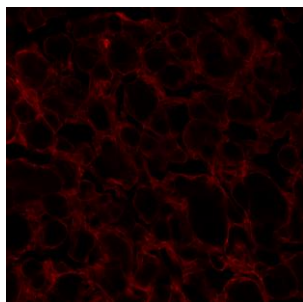
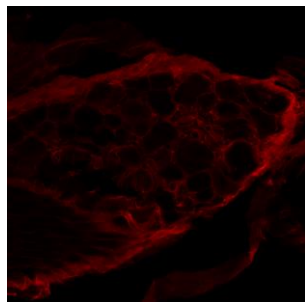
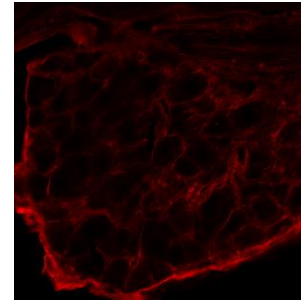
TG-F-R2275

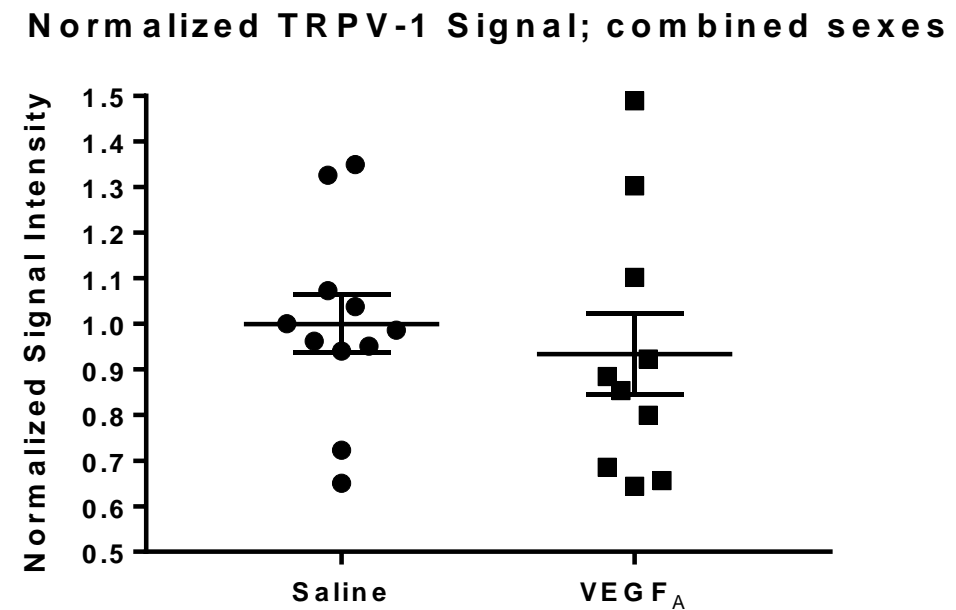
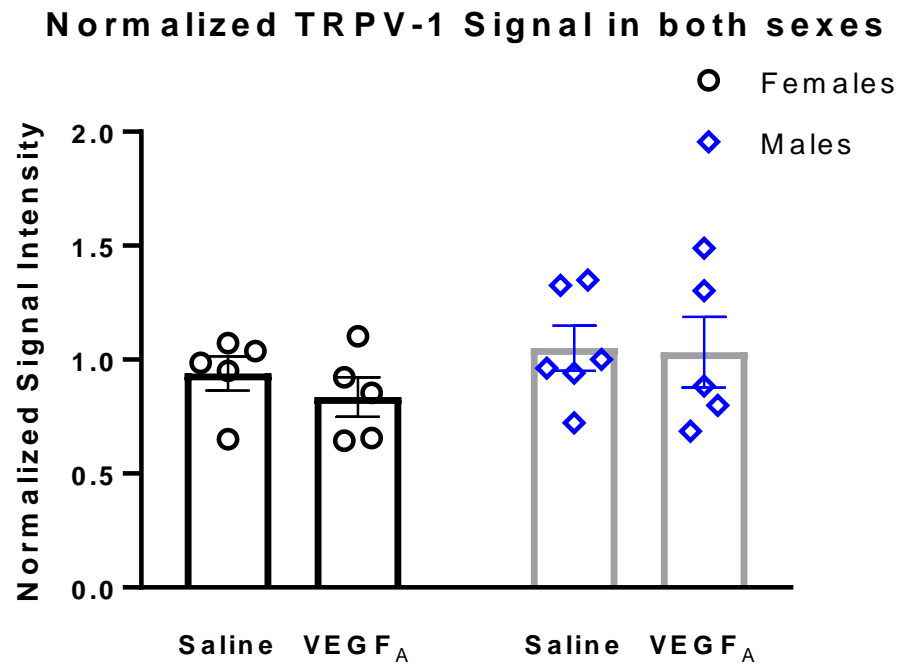


TG-F-R2499



TG-M-R2276





|             |    |   |   |
|-------------|----|---|---|
| Total count | 21 | M | F |
| Saline      | 11 | 6 | 5 |
| VEGF165     | 10 | 5 | 5 |



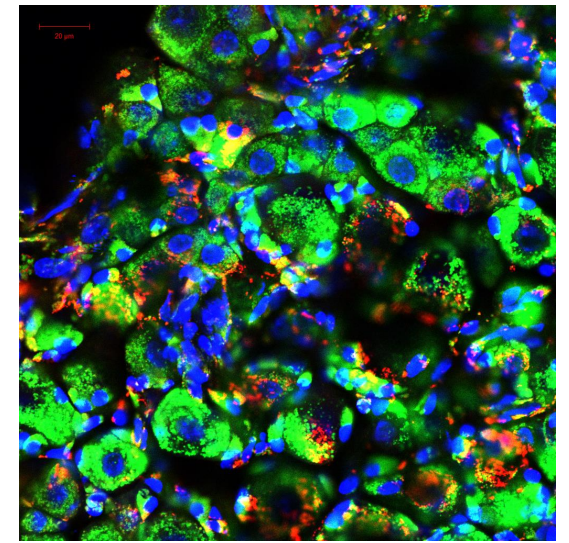
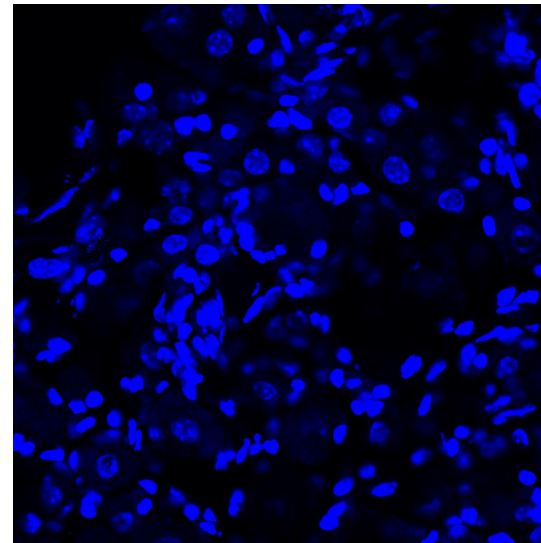
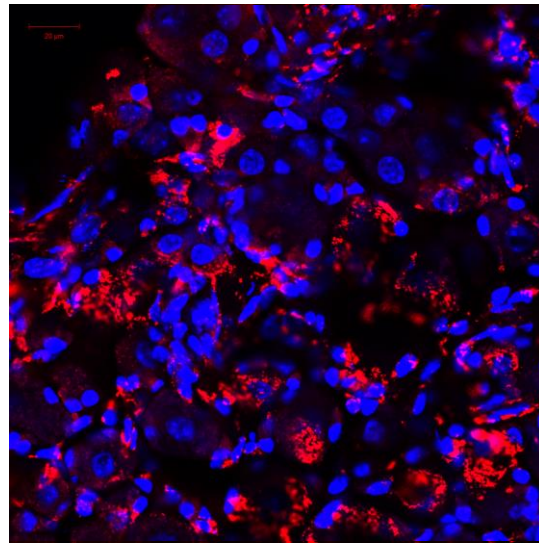
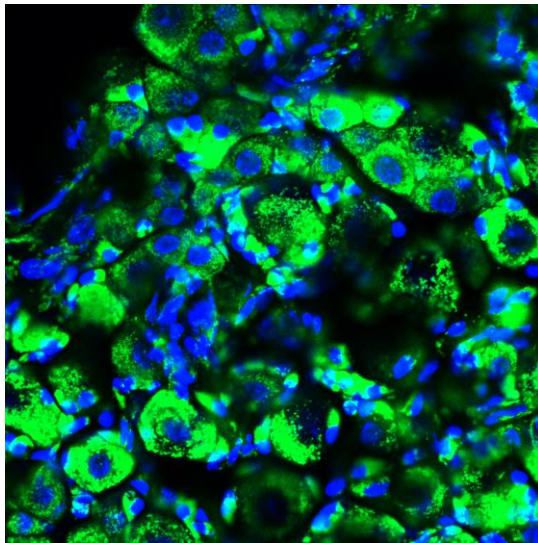
Antibody against TRPA1 exhibits signal in satellite glia cells and some DRG neurons.

TRPV1-ZsGreen +/+ mice  
(ZsGreen in 488)

TRPA1

DAPI

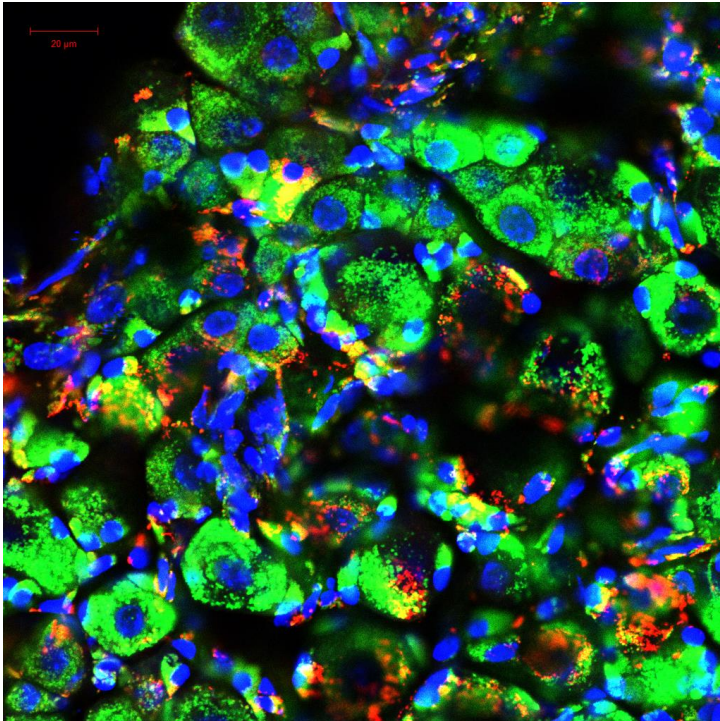
Overlay



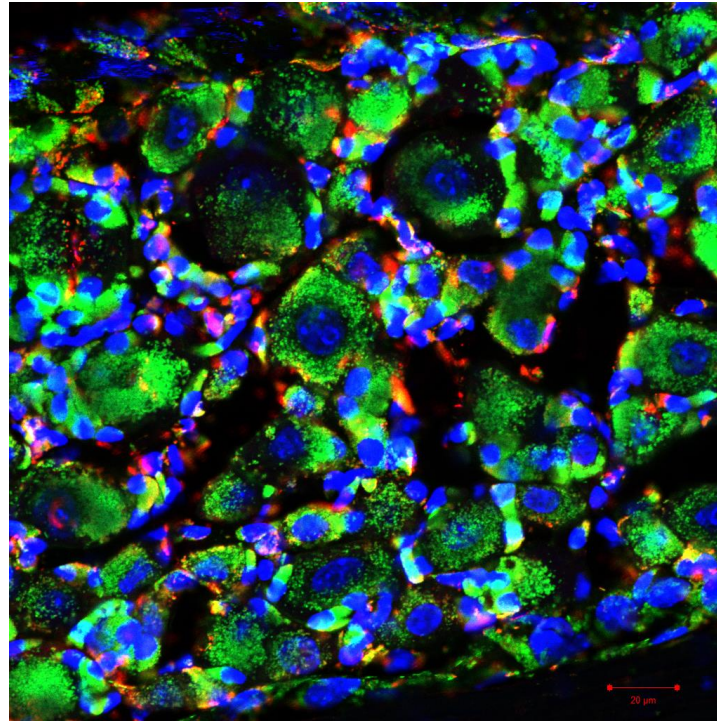


Antibody against TRPA1 exhibits signal in satellite glia cells and some DRG neurons.

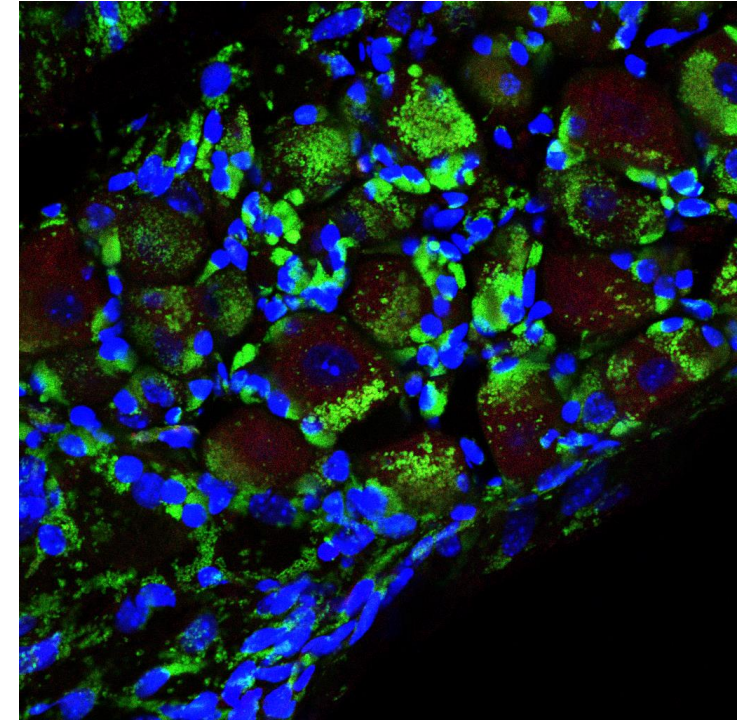
TG VEGF R2275



TG VEGF R2276



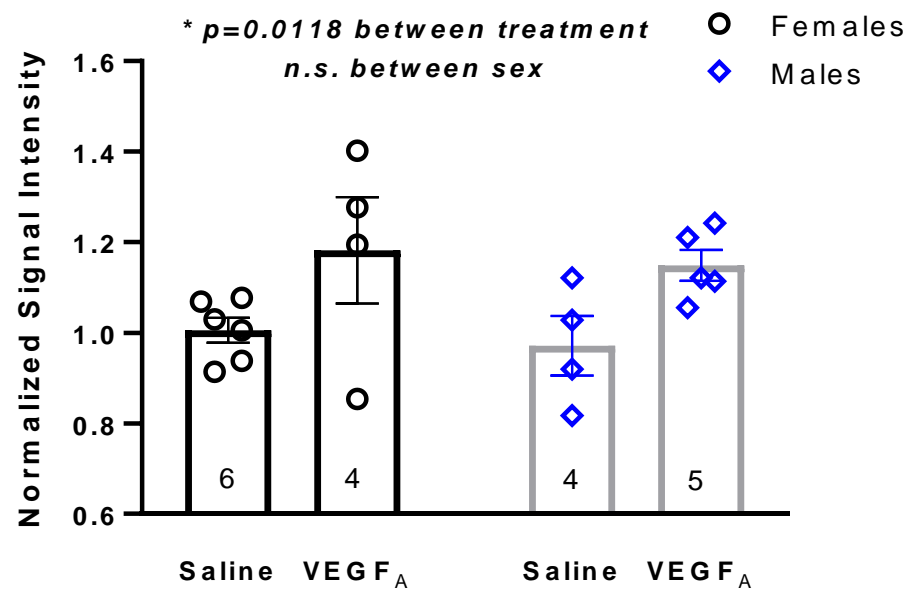
TG VEGF R2274 NP control



20190328 RGs/PBSVR1  
RG female\_R0165\_40x\_1  
Saline Instillation



### Normalized TRPA-1 Signal in both sexes



### Normalized TRPA-1 Signal; combined sexes

